INTRAVITAL STAINING OF DAMAGED LIVER CELLS

II. THE USE OF DYES IN THE STUDY OF NECROSIS AND REPAIR FOLLOWING ACUTE CHEMICAL INJURY [1]

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TWELVE FIGURES

INTRODUCTION

Intravital staining with acid diazo dyes has been used in the study of injury to several tissues and organs, including skeletal and cardiac muscle, the kidney, blood vessels, and the nervous and respiratory systems (see Cappell, '29a, b; and Williams, '47 and '49c for references). These dyes are excreted by the kidney and are stored as large granules in the macrophages of most tissues and organs, including those lining the sinusoids of the liver. They do not stain normal hepatic parenchymal cells of mice. Exposure to carbon tetrachloride, by ingestion, inhalation, or injection, damages the central one-third to one-half of hepatic lobules in most mammals (Edwards and Dalton, '42). Diazo dyes are then deposited in the cytoplasm of parenchymal cells located within that area and remain as a diffuse cytoplasmic stain for 72 hours (Willams, '48a, b). In this report the subsequent history of these vitally stained liver cells will be related.

METHODS

Sixty adult albino mice (average weight, 25 to 30 gm) were treated from one to 9 days with subcutaneous injections of

[1] This work was supported in part under the Commission on Liver Disease, Army Epidemiological Board, Preventive Medicine Division, Office of the Surgeon General; by research grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. P. H. S.; and by grants from the Medical Research Fund of the Graduate School of the University of Minnesota.

carbon tetrachloride (0.04 cm^3 daily in mineral oil) and acid diazo dyes (trypan blue or chlorazol fast pink 0.1 to 0.2 cm^3 of 0.5% aqueous solutions, daily). A majority of the mice were so treated for two to 4 days. Mice were killed at intervals of one to 9, 13, 21, 24, 29, 30, 33, 50, 65, 100, 140, 165 and 200 days subsequent to termination of treatment. Fifty-five mice received trypan blue; two, chlorazol fast pink; three, both dyes. Twelve mice were treated as above except that the sulphonated triphenylmethane dye, isamine blue, was substituted for a diazo dye and the mice were killed one to 16 days later. Fifty additional mice received carbon tetrachloride but no dye and were killed at similar intervals after treatment. Earlier studies had demonstrated that acid diazo dyes did not injure the liver in doses much larger than those used here (Williams and Hodge, '43; Williams, '48a).

Tissues were prepared for study by methods previously described in detail (Williams and Frantz, '48). For determination of cytoplasmic basophilia sections were stained in toluidine blue (5 to 10 drops of 0.5 toluidine blue in 20% alcohol added to 50 cm^3 of water) for 7 to 12 minutes. Routine hematoxylin and eosin preparations were also made.

The mice were fed an adequate diet of fox chow and no significant variations in weight occurred during the experiments. Food and water were available at all times. Injections of the dyes and of carbon tetrachloride were made between 9 and 10 A.M. and mice were killed at approximately the same time of day by manually crushing the cervical spinal cord. Mice dying during the course of the experiments are not included in the data presented here.

OBSERVATIONS

I. Hepatic injury, necrosis and repair following injection of carbon tetrachloride. Within 24 hours after the first injection of carbon tetrachloride parenchymal cells of the central zones were agranular and eosinophilic (fig. 1). A majority of the nuclei were basophobic. In sections stained with toluidine blue, loss of cytoplasmic basophilia was an

early and obvious indication of cellular injury. Cells of the middle zones were enlarged and showed a diminution in cytoplasmic basophilia. Cells of the peripheral (or portal) zones were unchanged except for cell to cell variations in the amount of cytoplasmic basophilia. In sections stained with hematoxylin and eosin the middle and peripheral zones appeared normal. These initial reactions were the same in mice treated with carbon tetrachloride for one or for 4 days. Continuation of such treatment for 5 to 9 days produced an increase in the number of enlarged and hypobasophilic cells within the middle and peripheral zones. Since a majority of the mice received the toxic compound for less than 5 days, however, the following account pertains to them.

Two to three days after termination of injection of carbon tetrachloride the centrilobular cells underwent necrosis. Nuclei were absent and cytolysis had occurred. During the next 14 days (figs. 3–6) the progressive changes in the central zones were (1) disintegration of the damaged cells, (2) fusion of remnants of parenchymal cells to form giant cells, (3) the appearance of histiocytic mononuclear cells in the area, and (4) phagocytosis of small cellular remnants and of the decomposing giant cells by these macrophages. Accompanying these changes there was hyperplasia of adjacent parenchymal cells which began within 48 hours after the initial injection of carbon tetrachloride. Mitoses were frequent among all of the midzonal cells including those which were enlarged and hypobasophilic. This cellular proliferation had repaired or replaced the central zone within 21 days (figs. 7 and 8). A few giant cells and large macrophages still remained in the area for 50 days. At 65 days the only residuum of injury was slight distortion of central sinusoids. Thirty-five days later all lobular zones were normal except for a small amount of stromal thickening around the central veins. The lobules showed no further change at the two later (140 and 200 days after cessation of treatment) intervals of study (fig. 12).

II. Intravital staining of the injured liver cells. Diffuse cytoplasmic vital staining of liver cells had the same localization as the injury described above. This staining remained unchanged for two to three days after cessation of injection of carbon tetrachloride and dye. During the next 4 days the cytoplasmic dye no longer appeared as a diffuse stain but was aggregated into large particles or granules (figs. 3, 4 and 10). These large particles were characteristic of the phase of cytoplasmic dissolution, remained attached to cytoplasmic remnants, and were present in giant cells for 21 days (figs. 5–8) and in macrophages during the 30th to 65th day interval. At the later time sinusoidal macrophages located outside of the former area of damage no longer contained dye. The remaining dye was present within the cytoplasm of large cells located in central zones. These macrophages did not line sinusoids and probably originated from histiocytes which appeared in the area during the earlier phase of cellular necrosis. No dye remained in livers of mice killed 100, 140 and 200 days after termination of treatment with dye and carbon tetrachloride (fig. 12).

A great majority of the vitally stained parenchymal cells followed the above pattern and underwent necrosis. An exception to this general pattern was observed in the liver of a mouse which was killed 7 days after termination of 4 days of injection of carbon tetrachloride and trypan blue. Necrosis of centrilobular cells had taken place in all lobules. Within these central areas evidence of vital staining consisted of large particles of dye within the giant cells and macrophages as described earlier. In a few of the lobules the middle zones (adjacent to the necrotic central zones) contained groups of parenchymal cells whose cytoplasm was still diffusely stained by trypan blue ("A" in figs. 9 and 10). In preparations stained with hematoxylin and eosin the nuclei were slightly hyperchromatic and the cytoplasm was agranular and orange (rather than the usual pink) in color. These cells had been injured at least 7 days previously and possibly as early as 11 days before (11 days had elapsed between the time of the

first injection of carbon tetrachloride and death of the animal). The quantity or intensity of the cytoplasmic vital staining was less than usual in the liver cells which subsequently underwent necrosis. Further, in all of the other mice diffuse cytoplasmic staining did not exist beyond the third day after termination of injection of trypan blue and of carbon tetrachloride. The prolonged phase of diffuse staining may indicate residual injury of a subnecrotic level since the cells were stained but still intact and relatively normal in appearance, or it may represent a retardation in the processes which will eventually lead to necrosis. This retardation may be related to the location of the cells (in the middle rather than the central zone). Any explanation based upon the possible vital staining of newly formed parenchymal cells does not seem tenable because of the absence of such a phenomenon in all of the other livers.

A few animals were treated with carbon tetrachloride for longer intervals, receiving it either daily or on alternate days for 9 days and were killed 24 hours later. In the group injected daily there were no completely normal cells. Vitally stained cells and necrotic cells were limited to the usual central area. Most of these central cells showed aggregation of dye into large particles which indicated injury incurred during the first 4 days of treatment. Others were diffusely stained (cytoplasm) as is common as early as 24 hours after a single injection of carbon tetrachloride (Williams, '48a). The cells of the middle and peripheral zones showed tremendous cytoplasmic swelling and greatly reduced nuclear and cytoplasmic basophilia, but were not vitally stained.

Total liver damage in the mice injected with carbon tetrachloride on alternate days over a 9 day period was no greater than in those treated daily with this toxic substance for one to 4 days. The livers differed from those of short-term treatment in that the diffuse cytoplasmic staining of some cells indicated recent damage while the presence of aggregated dye particles within other cells denoted injury which had occurred during the early part of the period of treatment.

The latter cells were obviously necrotic and numerous histiocytes were present in the adjacent area. Cells of the middle and peripheral zones were not vitally stained and were not necrotic.

The responses of damaged cells to each of the three dyes (trypan blue, chlorazol fast pink and isamine blue) were the same. Therefore the account of the history of cells stained by trypan blue will serve for the two other dyes. Simultaneous injection (at separate sites) of the two diazo dyes produced a combination of them in the cytoplasm of damaged cells with the result that the diffuse stain was purple instead of the blue of trypan blue or the pink of chlorazol fast pink.

DISCUSSION

The reactions of normal and damaged livers to the three dyes were identical in that damaged parenchymal cells were stained rather than normal ones and all three appeared as large cytoplasmic granules within macrophages. Trypan blue and chlorazol fast pink are acid diazo compounds which are almost indentical chemically, while isamine blue is a sulfonated triphenylmethane compound which has several properties in common with diazo dyes although structurally different. These are large molecular size, renal excretion and abundant deposition in all macrophages and within the epithelium of renal proximal convoluted tubules. Trypan blue is the most widely used intravital stain and needs no discussion. Isamine blue has been described by Cappell ('29a, b), and chlorazol fast pink by Williams and Hodge ('43).

Diffuse cytoplasmic staining of liver cells with intravitally administered dyes may be used as an indication of irreversible but prenecrotic cellular injury. These dyes do not become visible in normal liver cells, but when, as the result of injury, they have penetrated the cell's membrane and stained the cytoplasm, the severely damaged cells are unable to eliminate them, because of failure in excretory mechanism or of attachment or (binding) to cytoplasmic constituents. During subsequent phases of the reaction, the dye was aggregated into

particles and it appeared to remain bound to the disintegrating cytoplasm.

The true cytoplasmic staining observed here is different from the deposition of the dyes in granular form in epithelium of renal tubules, and the phagocytosis and intracytoplasmic segregation of dye particles by macrophages. In sectioned material the diffuse dye within damaged cells differs physically from that in macrophages and renal tubular epithelium in that it is very susceptible to solution by water and combines tinctorially with counterstains. In contrast the granular depositions are resistant to solution in water and do not combine with counterstains. When damaged cells reach the stage of necrosis, the diffuse staining no longer exists but has changed to an aggregated particulate form which is resistant to aqueous solutions and shows less reaction to counterstains (Williams and Frantz, '48). Another difference between diffuse cytoplasmic vital staining and granular deposition of dye is that in the former when cells are subjected to two vital dyes the resulting cytoplasmic staining will be a tinctorial combination of the two, while in the latter separate granules of different color occur (Williams and Frantz, '48).

Although the three dyes used here did not stain normal liver cells, other dyes do stain the hepatic parenchyma. Rose bengal, a phthalein or xanthene dye (Conn, '46), stains the cytoplasm of liver cells of mice and rabbits during the period of its excretion by the liver (Williams, '49a; Mendeloff, '49). A chemically closely related dye, fluorescein, stains liver cells under similar conditions (Grafflin, '47). A chemically different dye, the monoazo dye, azo rubin S, has been used to study hepatic excretory function in man (Rosenberg and Soskin, '40) and it stains the cytoplasm of liver cells of mice during the excretory phase (Williams, '50).

Two different approaches can be utilized in applying vital staining to the study of cellular injury. First, as in the present experiment, if the dye is not excreted by or does not normally enter or stain the cells in question, then the intra-

cytoplasmic presence of dye is atypical and indicates injury. Second, if the dye is excreted by the cells being studied, then the amount of vital staining and interval of staining become the criteria for ascertaining damage (Williams, '49a, b). In the second circumstance a prolonged retention of dye in the blood plasma may be associated with the protracted duration of cytoplasmic vital staining (Casals and Olitsky, '46).

A major purpose of this study was to test intravital staining as an indicator of the earliest manifestations of cellular injury. The dyes did indicate injury, but did so unequivocally only in the case of cells of which a majority subsequently became necrotic. The dyes normally excreted by the liver are superior as rapid and positive indicators of possible sublethal or reversible injury in which instance the time required for complete clearance, as determined by intravital staining of the cytoplasm of the involved cells, becomes the criterion of injury (Williams, '49a, b).

Cytologically the first evidence of hepatic parenchymal reaction to injury was a decrease in cytoplasmic basophilia which seemed to result from a diminution in ribopolynucleotides (Williams and Greenberg, '50a, b). Changes in cytoplasmic nuclei acid content as concomitants or manifestations of cellular activity and injury have been considered by many workers of whom the cited references represent only a few (Lison, '36; Deane, '46; Opie, '46, '47a, b, and '48; Opie and Lavin, '46; Davidson, '47; Szanto and Popper, '48; Lagerstedt, '49). Another early indication of injury to liver cells is the occurrence of a positive cytoplasmic alkaline phosphatase reaction (Williams and Greenberg, '50a, b). Careful histochemical studies of injured livers have demonstrated that considerable cytoplasmic alteration may take place without the occurrence of atypical vital staining of the cytoplasm. This was shown in rats receiving a high fat-low protein diet. The hepatic parenchymal cells lost their basophilia and contained large amounts of fat, but were not stained by trypan blue (Williams and Molander, '50). However, it seems clearly

demonstrated that vital staining of the cytoplasm of hepatic parenchymal cells by acid diazo dyes does occur in the presence of injury sufficient to produce necrosis.

SUMMARY

In mice the centrilobular hepatic cells injured by carbon tetrachloride were stained intravitally by two acid diazo dyes and by the sulphonated triphenylmethane dye, isamine blue. The staining of these cells was the cytoplasmic suffusion type, not granular deposition. These central cells were also very hypobasophilic (nuclei and cytoplasm). Many cells of the middle zones also showed loss of cytoplasmic basophilia but were not vitally stained.

Subsequent necrosis was limited to vitally stained cells of the central zones. Cytolysis occurred within two to three days after termination of treatment with carbon tetrachloride at which time the cytoplasmic vital stain was aggregated into large particles or granules. These accumulations of dye remained attached to cytoplasmic remnants for as long as such were identifiable. The cytoplasmic remnants and the adhering dye became parts of fusion-type giant cells and were eventually phagocytosed by macrophages.

Mitoses of midzonal cells occurred as early as 48 hours after injection of carbon tetrachloride. These new cells replaced the necrotic central zones, and repair without fibrosis was essentially complete within 21 to 30 days.

The damaged central cells reacted in the same fashion to each of the three dyes. These dyes did not stain the cytoplasm or nuclei of parenchymal cells of normal livers although they were abundantly deposited as segregated granules in in adjacent sinusoidal macrophages. The dyes served as adequate labels for hepatic cells irreversibly injured by carbon tetrachloride.

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livers of rats to an acid diazo dye. Federation Proceedings, *9:* 136.

EXPLANATION OF PLATES

The livers shown in the following plates were fixed in an aqueous alcohol-formalin-acetic acid solution and were sectioned in paraffin at 10 μ. The intravital staining was by trypan blue. Photographed in carbon arc illumination with a green filter.

PLATE 1

EXPLANATION OF FIGURES

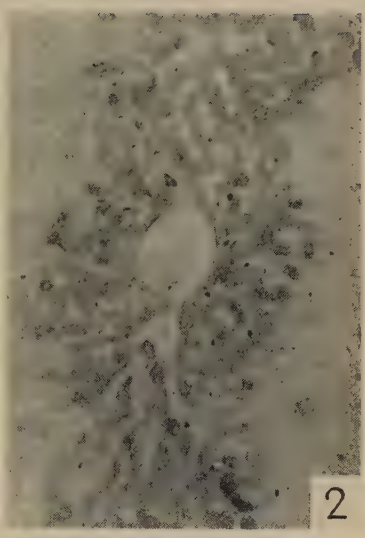
1 Centrilobular area of injury 24 hours after two daily injections of carbon tetrachloride. Stained with hematoxylin and eosin. $\times$ 150.

2 Same section as shown in figure 1. No counterstain. Note cytoplasmic staining of injured central cells by trypan blue. $\times$ 150.

3 One week after cessation of 4 days treatment with carbon tetrachloride. Observe necrosis of central cells, appearance of histiocytic cells, aggregration of dye into large granules and fusion of cells. H. and E. $\times$ 150.

4 Uncounterstained section of same liver as above. In upper portion of the figure and lower area to the right observe aggregation of trypan blue into granules. The middle area of the photograph contains essentially normal parenchymal cells, and numerous sinusoidal macrophages filled with dye (the dark objects). $\times$ 150.

1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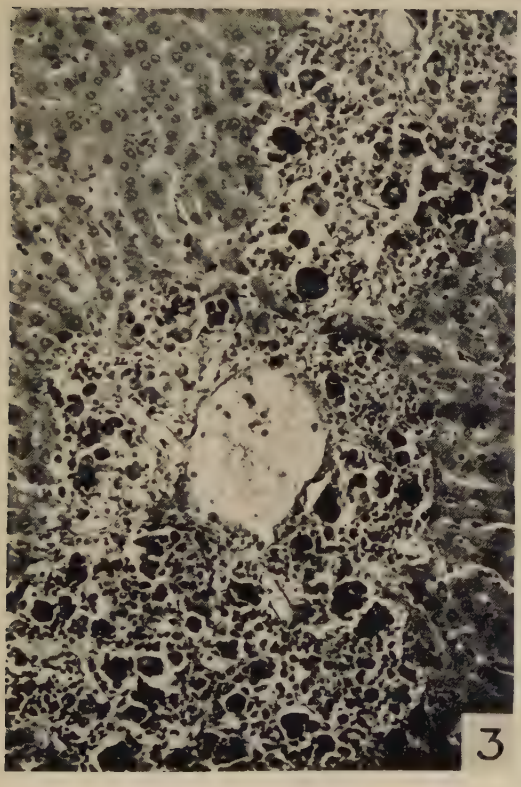
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PLATE 2

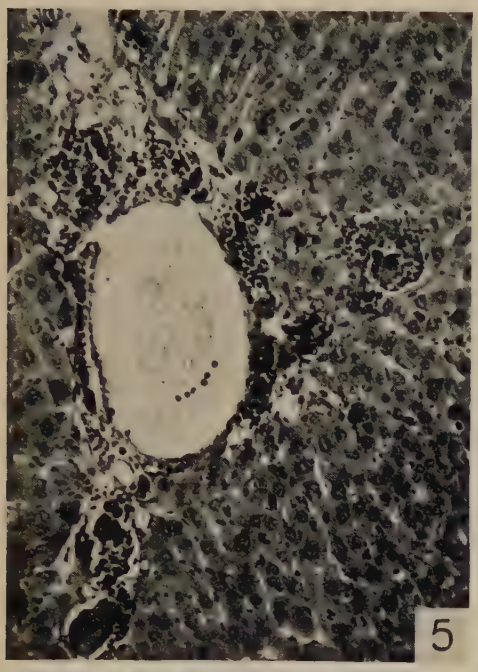
EXPLANATION OF FIGURES

5 Centrilobular area 13 days subsequent to carbon tetrachloride-treatment. The dark masses are giant cells formed by fusion of necrotic, dye-containing parenchymal cells. New liver cells have replaced about one-half of the area of necrosis. Hematoxylin and eosin. × 150.

6 Section of same area shown above. No counterstain. Granules of trypan blue in giant cells. × 150.

7 Twenty-one days after carbon tetrachloride treatment. Central area repaired. Trypan blue remains in giant cells and macrophages. × 150.

8 Central area from same liver as in figure 7, but showing less dye remaining. No counterstain. × 150.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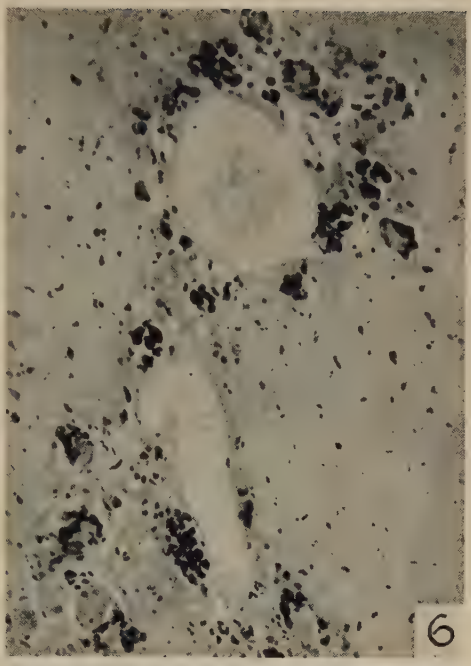
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PLATE 3

EXPLANATION OF FIGURES

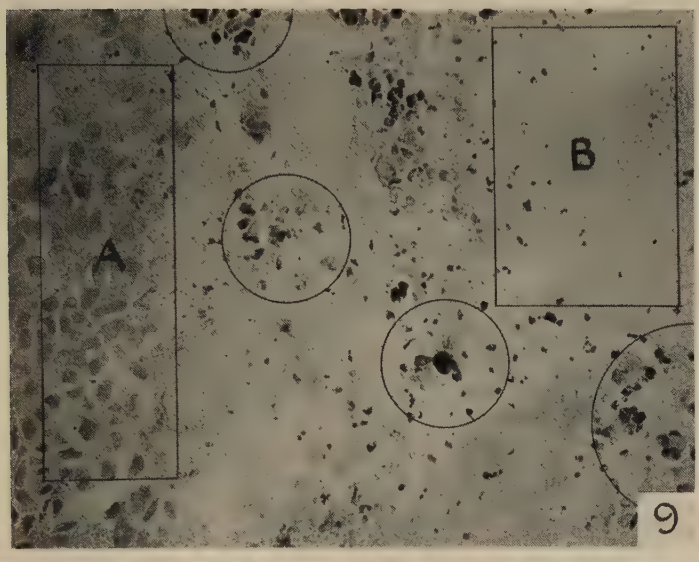
9 Uncounterstained section of liver 7 days subsequent to termination of 4 days of carbon tetrachloride treatment. × 150.

A: Hypobasophilic parenchymal cells still showing uniform intravital staining of cytoplasm.

B: Ingrowth of normal cells into damaged area.

Circles: Contain parenchymal cells showing necrosis and aggregation of the cytoplasmic vital stain into particles.

10 Same as above, hematoxylin and eosin. × 500.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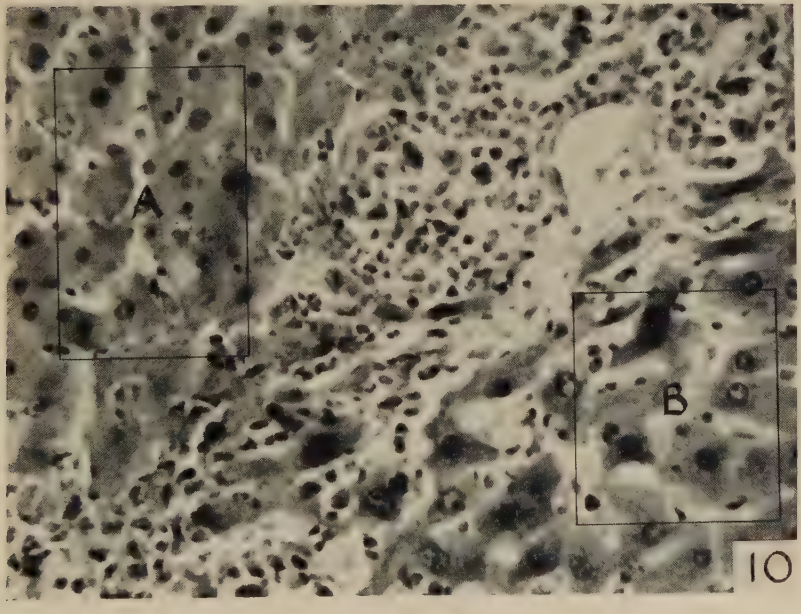
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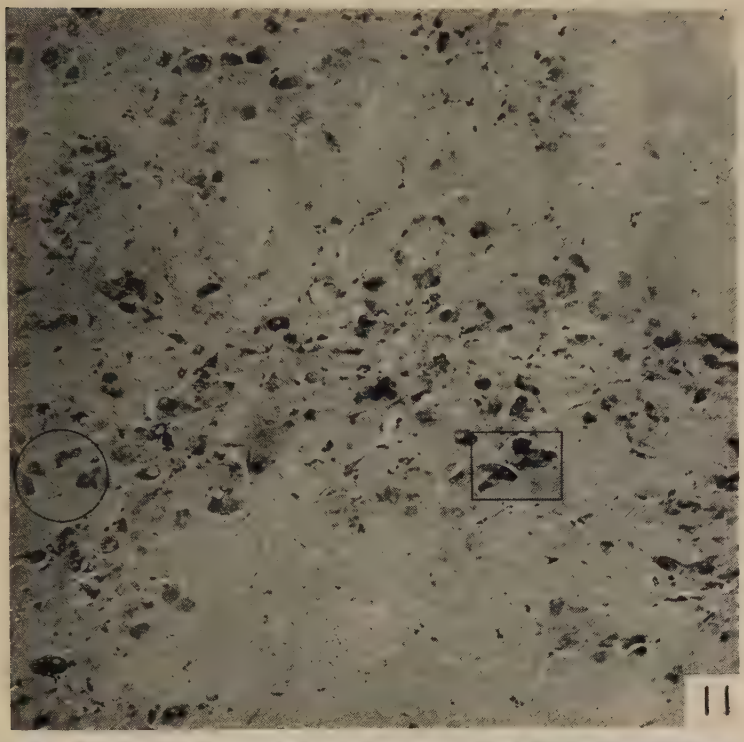
PLATE 4

EXPLANATION OF FIGURES

11 Twenty-four hours subsequent to termination of treatment with carbon tetrachloride on alternate days over a period of 9 days. Section was counterstained for a few seconds in an acetone solution of neutral red. Areas similar to that within the *circle* show recently damaged parenchymal cells whose cytoplasm is lightly and evenly stained with intravital trypan blue. Areas similar to that within the *rectangle* contain cells damaged earlier and which show necrosis and aggregation of trypan blue into large granules (dark masses). × 150.

12 Liver at 140 days after two days treatment with carbon tetrachloride. Complete repair has taken place and there is no evidence of vital staining. × 150.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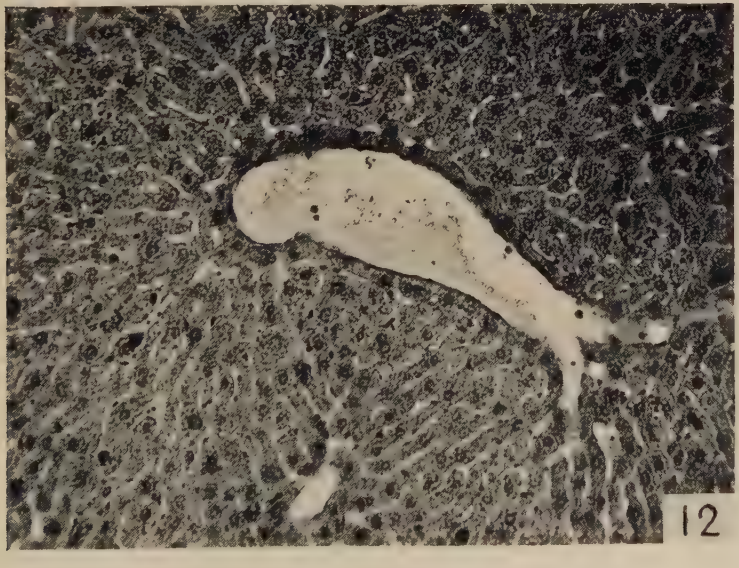
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THE EFFECT OF ESTROGEN ON THE ALVEOLAR BONE AND TEETH OF MICE AND RATS [1]

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SEVEN FIGURES

INTRODUCTION

A number of studies have shown that in the mouse estrogen retards the proliferative phase in the growth of the epiphyseal and condylar cartilage, but increases the amount of spongy bone in the metaphysis and diaphysis (Gardner and Pfeiffer, '38 and '43; Day and Follis, '41; Silberberg and Silberberg, '46; Urist, Budy and McLean, '48).

The present study deals with the changes in the dental supporting structures following the injection of α-estradiol benzoate in mice and rats. As far as we were able to ascertain, such investigations have not previously been reported. Since it has been claimed (Silberberg and Silberberg, '46) that estrogen accelerates certain age changes, it seemed advisable to compare the age changes in the alveolar bone of the mouse and rat with those produced by estrogen.

REVIEW OF THE LITERATURE

It is well established that the administration of large doses of estrogen produces osteosclerosis in the skeleton of the rat

[1] This investigation was supported (in part) by a research grant from the National Cancer Institute of the National Institute of Health, United States Public Health Service.

[2] Part of a dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Histology at the University of Illinois.

and mouse (Gardner and Pfeiffer, '38 and '43; Day and Follis, '41; Urist, Budy and McLean, '48). In the mouse the osteosclerosis is a result of increased endosteal bone formation and inhibition of physiologic resorption of bone (Gardner and Pfeiffer, '43; Urist, Budy and McLean, '48), whereas in the rat the osteosclerosis is merely a result of inhibition of the physiologic resorption of newly formed bone (Urist, Budy, and McLean, '48). In both rats and mice, the administration of estrogens retarded the growth of the long bones (Day and Follis, '41), and increased their breaking strength (Gardner, '43). The response to estradiol administration varied with age, sex and strain (Gardner and Pfeiffer, '43; Silberberg and Silberberg, '46).

MATERIAL

This study is based on the histologic investigation of the effects of α-estradiol benzoate [3] upon the supporting dental structures of three groups of mice and three groups of rats. The experimental procedures were conducted in the laboratory of Dr. F. C. McLean of the University of Chicago.

Mice

All mice were of CFI strain (Carworth Farms, Rockland County, New York). Both males and females were used.

Group A. Six litter-mates ranging in weight from 16 to 20 gm were used as controls. They were given injections of corn oil (table 1).

Group B. Thirty-three normal mice, ranging from 6 days to 30 months of age, were studied in order to determine whether the normal age changes of the supporting dental structures were in any way similar to the changes following the injections of α-estradiol benzoate injections.

Group C. Seventeen mice varying in weight from 16 to 24 gm at the start of the experiment were given injections of

[3] Alpha estradiol benzoate was procured from Roche Organon, Inc., Nutley, New Jersey.

α-estradiol benzoate. The amount of α-estradiol benzoate injected varied from 1 mg to 11.5 mg over a period of one to 8 weeks (table 2). The maximum dose did not exceed 2 mg per week. Each dose contained 0.5 mg of α-estradiol benzoate in 0.05 ml of commercial grade corn oil, and was given intramuscularly in the hind legs. The injections were discontinued when toxic symptoms appeared. The usual symptoms of toxicity were diarrhea, loss of appetite and abdominal distension.

TABLE 1

Experimental data and findings in the upper molars of 6 litter-mate control mice which were given injections of corn oil (Group A)

NO.	NUMBER OF INJECTIONS	DURATION OF EXPERIMENT	AVERAGE WIDTH OF BORDER OF NEW ENDOSTEAL BONE IN μ	AVERAGE WIDTH OF EPITHELIAL RESTS IN μ
		weeks		
105	4	1	0	11
113	1	2	0	10
117	0	4	0	10
121	4	4	0	12
125	16	8	0	12
129	8	8	0	7

All mice were sacrificed and autopsied one week after the final dose of estradiol.

Rats

All rats were of a pure Wistar strain. Both males and females were used.

Group A. Controls. Two rats, 290 and 343 days of age were given daily intramuscular injections of 0.1 ml of corn oil and were sacrificed 4 weeks after the start of the injections. Four rats, 26 to 29 days of age were given 4 to 32 injections of 0.1 ml corn oil and sacrificed 29 to 62 days after the first injections (table 3).

Group B. Ten rats, ranging in age from 287 to 343 days were given daily intramuscular injections of 1.0 mg of α-estradiol benzoate in 0.1 ml of corn oil. Two were sacrificed after one week, and 8 were sacrificed after 4 weeks of estradiol administration (table 3).

TABLE 2

Experimental data and findings in upper molars of 17 mice which were given injections of α-estradiol benzoate (Group C)

NO.	NUMBER OF INJECTIONS	DURATION OF EXPERIMENT	TOTAL AMOUNT OF ESTRADIOL	AVERAGE WIDTH OF BORDER OF NEW ENDOSTEAL BONE IN μ	AVERAGE WIDTH EPITHELIAL RESTS IN μ
		weeks	*mg*		
103	2	1	1	19	12
114	2	2	1	39	14
104	4	1	2	11	14
108	4	1	2	42	..
111	4	2	2	49	11
115	4	2	2	39	12
118	4	4	2	50	12
122	4	4	2	68	14
127	4	8	2	64	13
128	6	8	3	46	13
130	7	8	3.5	31	12
112	8	2	4	31	20
116	8	2	4	62	17
123	8	4	4	62	19
131	15	8	7.5	50	12
124	16	4	8	54	8
132	23	8	11.5	49	13

Group C. Thirteen rats, 26 to 29 days of age, received from one to 31 injections of α-estradiol benzoate. At each administration, 1.0 mg of α-estradiol benzoate in 0.1 ml of corn oil was injected intramuscularly. The rats were sacrificed at 7 to 65 days after the start of the experiment (table 3).

METHODS

Histologic preparations. All animals were decapitated immediately after sacrifice and the heads were bisected in the mid-sagittal plane. Specimens were then fixed in Zenker's

solution (4% formaldehyde) (Maximow's modification), decalcified in 5% nitric acid, embedded in celloidin, sectioned and stained with hematoxylin and eosin or impregnated with silver. Central sections of the maxillary and mandibular first molars, cut mesiodistally and central longitudinal sections through the incisors were selected as representative areas.

TABLE 3

Data on the control and experimental rats

NUMBER		AGE AT THE BEGINNING OF THE EXPERIMENT	AGE AT AUTOPSY	NUMBER OF INJECTIONS [1]
		in days	*in days*	
EB 401		290	318	28
EB 409		343	371	28
EB 13	Group	26	88	4
EB 29	A	27	87	8
EB 37		26	55	8
EB 61		29	95	32
EB 402		287	294	7
EB 403		317	345	28
EB 404		290	318	28
EB 405		317	345	28
EB 406	Group	317	345	28
EB 407	B	290	297	7
EB 408		289	317	28
EB 410		343	371	28
EB 411		343	371	28
EB 412		343	371	28
EB 3		26	34	2
EB 12		26	88	4
EB 14		26	82	1
EB 18		27	41	2
EB 19		27	42	4
EB 20	Group	27	46	8
EB 36	C	26	58	16
EB 38		26	54	4
EB 43		26	83	8
EB 48		26	87	16
EB 50		26	83	8
EB 55		29	86	16
EB 60		29	94	31

[1] Injections given to group A (control) consisted of 0.1 ml of corn oil and those given to groups B and C (experimental) consisted of 1.0 mg of α-estradiol benzoate in 0.1 ml of corn oil.

Alveolar bone. The alveolar bone was studied in relation to: (a) apposition and resorption; (b) size of the bone marrow spaces in the inter-radicular septum; and (c) type of bone marrow.

The bone deposited after the injection of α-estradiol benzoate showed a distinctive staining quality when stained with hematoxylin and eosin. Thus, the amount of new bone deposited in the marrow cavities could be measured. The thickness of new bone in 4 representative areas was determined for each affected animal, and the average was recorded.

Height of crest of alveolar bone. The height of the alveolar crest in the molar region was classified in three stages: (a) in the cervical third of the root; (b) in the middle third of the root; and (c) in the apical third of the root. The distance between the cemento-enamel junction and the alveolar crest was also measured.

Periodontal membrane. The periodontal membrane was studied for possible age changes and the width of the periodontal membrane was measured by means of a micrometer. Two measurements were taken in the middle third of the mesial and distal surfaces of each root.

Cementum. The cementum was studied for changes with increasing age. Its thickness was measured in the middle third of the mesial and distal surfaces of the roots.

Epithelial structures. The epithelial structures were studied with regard to: (a) changes in hornification of the gingiva; (b) changes in the sites of the epithelial attachments; (c) prevalence and size of the epithelial remnants.

FINDINGS

Histology of the supporting structures of the first molars of normal mice

(Groups A and B)

Alveolar bone. At 20 days of age (fig. 2) the alveolar crest was about 10 μ apical to the cemento-enamel junction. As a

rule, the alveolar sockets of the single roots showed resorption on the surfaces facing mesially, and apposition on the surfaces facing distally indicating a distal drift of the molars. Because of the divergence of some roots, this picture was modified in some instances. The alveolar crest and the fundic areas showed bone apposition.

The pattern of bone apposition and resorption did not change with increasing age, but the reduced number of osteoblasts seemed to indicate less bone apposition in older animals. With increasing age, the distance between the alveolar crest and the cemento-enamel junction increased. At 30 months of age, the alveolar crest was about 0.6 mm apical to the cemento-enamel junction or in the apical third of the root (fig. 3).

In the younger animals, the bone marrow space was divided into a number of compartments by bone trabeculae. Most endosteal surfaces showed resorption of bone. In the older animals, a uniform marrow space, slightly increased in size, was present. The endosteal surfaces showed neither apposition nor resorption (aplastic). The bone marrow in the 20-day old mouse was of the hemopoietic type (fig. 2). With increasing age, the hemopoietic bone marrow was partially replaced by fatty marrow.

Periodontal membrane. The structure and width of the periodontal membrane did not appear to change with age. The average width of the periodontal membrane of the maxillary and mandibular first molars was 65 μ, and ranged from 45 μ to 95 μ in the maxillary, and from 55 μ to 85 μ in the mandibular first molar.

Cementum. The first maxillary molar of the mouse was erupted at 20 days. At this time, the length of the mesial root measured from the cemento-enamel junction to the apical border of the dentin was 0.9 mm, and the distal buccal root was 0.8 mm in length. The cementum was cellular. It measured in thickness an average of 3 μ in the middle of the root and seemed evenly distributed on all surfaces. Cementoblasts lined the cemental surface in almost all areas with the ex-

ception of the apex, where no cementum was found (fig. 2). In animals older than 20 days of age, dentin apposition at the apical surface did not contribute to the increase of the root length, but the root increased in length by cementum apposition.

The thickness of the cementum increased with age (fig. 3), so that at 50 days it measured an average of 6 μ; at 94 days an average of 7 μ; and at 30 months an average of 30 μ in the middle third of the root. With advancing age, the cementum showed a marked increase in thickness at the apical region (fig. 3).

Changes of supporting structures of the first molars of the mice treated with α-estradiol benzoate

(Group C)

A characteristic response to the injection of α-estradiol benzoate was observed in the bone marrow spaces of all experimental mice; both males and females were equally affected (fig. 1).

Alveolar bone. The size of the medullary cavity was decreased as a result of apposition of new bone and lack of resorption (figs. 4–7). Following large doses of estradiol, new bone was also deposited within the Haversian canals (fig. 7).

The newly deposited bone, as compared with the pre-experimental bone, stained darker with hematoxylin and lighter with silver impregnation. These characteristics of the experimental bone indicated fewer fibrils in its matrix. The number of osteocytes per unit area appeared to be decreased about one-third to one-half the number found in the adjacent pre-experimental bone. The increased thickness of new bone apposition ranged from 11 μ to 68 μ in width (table 2).

This increase in bone was proportional to the increase in the estradiol dose up to 2 mg per week. The latter dose seemed to produce the maximum effect.

Fig. 1 Diagrammatic representation of the maxillary first molar of mouse showing normal medullary cavity and the changes that take place following the injection of α-estradiol benzoate. (A) Normal medullary cavity (MC). (B) Moderate response to α-estradiol benzoate. Note the new bone in the medullary cavity (MC). (C) Severe response to α-estradiol benzoate. The medullary cavity (MC) is almost obliterated.

α-Estradiol benzoate given in a single large dose caused less new bone deposition than when the same dose was administered in smaller units and injected over a prolonged time (table 2).

The pattern of apposition and resorption at the periodontal surface of the alveolar walls was the same in the control and experimental animals.

Periodontal membrane. The structure and width of the periodontal membrane of the estrogen-treated mice did not differ from the normal. The epithelial remnants, however, appeared to be increased in number and size in the experimental animals. They varied in number from 0 to 16, with an average of 5.5 rests in each section, while the control group showed a range of one to 6 epithelial remnants with an average of three rests per section. The size of the remnants varied from 8 μ to 20 μ in the experimental group (table 2), and from 7 μ to 12 μ in the control group (table 1).

Cementum. The cementum of the estrogen-treated mice had the same thickness as that found in the control group at similar age levels. It measured an average of 6 μ in the middle of the root and seemed evenly distributed on all surfaces. The cementum appeared cellular.

Epithelial attachment. The epithelial attachment extended to comparable levels in the normal and experimental animals. No changes were found in the structure of the gingival epithelium.

Tooth structures. The structures of the enamel, dentin, cementum and pulp were not affected by α-estradiol benzoate.

Histology of the supporting structures of the maxillary incisors of normal mice

(Groups A and B)

Alveolar bone. The alveolar bone of the maxillary incisor is formed by the maxilla and premaxilla. The premaxilla contributes the entire lingual part and the anterior two-thirds of the labial part of the incisal socket. The maxilla forms the

entire fundus and the posterior one-third of the labial alveolus. In a longitudinal section, the premaxillo-maxillary suture is found on the lingual surface near the fundus, and on the labial surface at a plane slightly anterior to the lingual portion of the suture.

At 20 days of age, the maxillary incisor of the mouse was erupted. At this time, the fundic portion of the maxilla and the labial alveolar surface of the premaxilla showed resorption. The lingual alveolar surface of the premaxilla and the labial alveolar surface of the maxilla showed apposition. Although with increasing age bone apposition and resorption, as indicated by the number of osteoblasts and osteoclasts, became less active, the pattern in which the alveolar bone was laid down or resorbed remained unchanged. Some areas of previous resorption exhibited apposition to allow for attachment of periodontal fibers.

The marrow spaces of the lingual portion of the premaxilla did not change in size with increasing age. The marrow which at 20 days was hemopoietic was replaced by fatty marrow with advancing age.

Changes in supporting structure of the maxillary incisor of mice treated with α-estradiol benzoate

(Group C)

Endosteal apposition of bone increased in amount and led to reduction in the size of the medullary cavities of the lingual portion of the premaxilla. This new bone had the same appearance and characteristics as the new bone found in the medullary cavities of the alveolar bone of the molars.

The other supporting structures of the maxillary incisor and the enamel, dentin, cementum, and pulp were not affected by the injection of α-estradiol benzoate.

Supporting structures of the incisors and first molars of rats

Comparison of control rats and rats injected with α-estradiol benzoate showed no significant differences in the alveolar

bone, in the size of the bone marrow spaces, in the periodontal membrane, or in the gingiva.

DISCUSSION

Response of bone. The most striking change following the injection of α-estradiol benzoate into mice was an osteosclerosis of the alveolar bone, which was confined to endosteal bone formation (fig. 7). The bone deposited after the injection of α-estradiol benzoate was different from the structure of pre-experimental lamellated bone. A unit of newly deposited bone contained fewer osteocytes and, therefore, more intercellular substance than did a comparable unit of lamellated bone. In addition, the intercellular substance, as observed by silver impregnation, contained fewer fibrils and more cementing substance. Since only the cementing substance is calcified and since the experimental bone showed an increase of cementing substance per unit area, it may be concluded that the experimental bone contained a greater amount of mineral salts than normal bone. The higher concentration of inorganic substances in the bones of estrogen-treated mice was already pointed out by Wentworth, Smith and Gardner ('40) and Gardner and Pfeiffer ('43), who studied the ash content of normal and estrogen-treated bones.

The bone deposited after α-estradiol benzoate injections also differs from immature bone. In sections stained with silver, the former stains light while the latter appears very dark. The fact that the immature bone is highly cellular and stains dark with silver indicates that it contains large numbers of bundles of coarse fibrils and relatively little intercellular substance. Thus, it contains less mineral salts than both the mature bone (Wentworth, Smith and Gardner, '40) and the bone deposited after α-estradiol benzoate injections.

One may, therefore, speculate that the administration of estrogen influences the formation of a type of bone which is specifically adapted to store mineral salts. One of the physiologic functions of estrogen might be the storage of mineral salts to be used in the supply of minerals for the cal-

cification of egg shells in birds (Bloom, Bloom and McLean, '41), or the calcification of the fetal skeleton. It must be noted that this bone is deposited only in the medullary cavities, and that the injection of α-estradiol benzoate did not change the apposition-resorption pattern of the surface of the alveolar bone. By this arrangement, a future need for mineral salts by the organism could be satisfied without disturbing the functional architecture of the bone.

The new bone deposited in the alveolar process had the same appearance and seemed to react with the same intensity as the new bone deposited in the medullary cavity of the femur (Urist, Budy and McLean, '48). On the other hand, the more specifically dental tissues, such as enamel, dentin, pulp and cementum did not show any response to estradiol benzoate. The lack of changes in the dental structures is not surprising, since these structures, unlike bone, are not storehouses of calcium.

Responses of epithelium. The injections of α-estradiol benzoate caused a proliferation of the epithelial remnants in the periodontal membrane which appeared to have increased in number and size. However, no changes could be found in the hornification of the oral epithelium in the experimental group. This is contrary to the findings of Ziskin et al., ('36), who reported an increase in hornification in estrogen-treated monkeys. The difference in reaction is probably due to the difference of the mucous membrane in the two species of animals. The mouse and the rat, in contrast to the monkey, normally show a high degree of hornification of the mucous membrane.

Lack of response in the alveolar process of rats. The absence of any reaction to α-estradiol benzoate in the supporting tissues of the teeth of the rat is in agreement with previous observations on long bones. It has been shown that accumulation of bone tissue in the metaphyses of the tibia and femur following administration of α-estradiol benzoate is due to a decreased resorption rather than an increased ap-

position. Since normally endosteal resorption plays only a minor role during the growth of the alveolar process, it is not surprising that the alveolar bone in rats did not show any changes following the administration of α-estradiol benzoate.

Comparison of normal age changes in the alveolar bone with experimental changes

In mice the administration of α-estradiol benzoate leads to a decrease in the size of the medullary cavity of the alveolar bone by apposition of a specific and typical bone tissue. The alveolar bone of aged mice showed no resemblance to the changes following estradiol injections. The marrow spaces of older mice were even slightly larger than those of younger mice.

SUMMARY AND CONCLUSIONS

This study was based on a histologic examination of the teeth and supporting dental structures of three groups of mice and three groups of rats. Seventeen mice, ranging in weight from 16 to 24 gm at the beginning of the experiment, were given injections of α-estradiol benzoate varying from 1 mg to 11.5 mg over a period lasting from one to 8 weeks. The maximum dose did not exceed 2 mg per week. Six male litter-mates were used as controls. In addition, 33 normal mice, ranging from 6 days to 30 months of age were studied in order to determine whether the normal age changes of the alveolar bone were in any way similar to the changes following α-estradiol benzoate injections.

Ten mature rats varying in age from 287 to 343 days were given a total dosage of 7 to 28 mg of α-estradiol benzoate, and 13 young rats between 26 and 29 days of age were given a total dosage of 1 to 31 mg of α-estradiol benzoate. Six rats of comparable age were used as controls.

The supporting tissues of the molars of the rats did not show any significant changes.

In the supporting tissues of the teeth of the mice, the following observations were made:

1. Injections of α-estradiol benzoate led to an osteosclerosis of the alveolar bone. It was confined to the medullary cavities and consisted of apposition of a specific type of bone tissue. The structure of the latter indicated an increased mineral content.

2. The amount of new bone was proportional to the amount of α-estradiol benzoate injected. A dose of 2 mg per week seemed to have the maximum osteogenic effect.

3. Repeated injections of a smaller dose of α-estradiol benzoate resulted in a greater bone deposition than a single, large dose.

4. Enamel, dentin, cementum, pulp and gingiva were not affected by α-estradiol benzoate injections.

5. A slight increase in number and size of the epithelial rests was noted in the estradiol-treated animals.

6. Males and females were equally susceptible to α-estradiol benzoate.

7. The findings indicated that the changes in the alveolar process produced by injections of α-estradiol benzoate were not comparable to the age changes of the alveolar process.

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PLATE 1

EXPLANATION OF FIGURES

2 Photomicrograph of a mesio-distal section of the first maxillary molar of normal mouse, ScM 10, 20 days of age. Hematoxylin-eosin stain (× 42). Note the thin layer of cementum (c), the alveolar crest (ac) in the region of the cervical third of the root, the apical end of the epithelial attachment (ea) at the cemento-enamel junction, the hemopoietic bone marrow (bm), pulp (p), dentin (d), enamel space (es).

3 Photomicrograph of a mesio-distal section of the first maxillary molar of normal mouse, Mt 6, 30 months of age. Hematoxylin-eosin stain (× 42). Note marked cementum apposition (c), alveolar crest (ac) in the apical third of the root, bone marrow containing hemopoietic and fatty tissue (bm), the apical end of the epithelial attachment (ea) on the cementum, pulp (p), dentin (d), and the enamel space (es).

bm
c
p
ac
ea
d
es
2

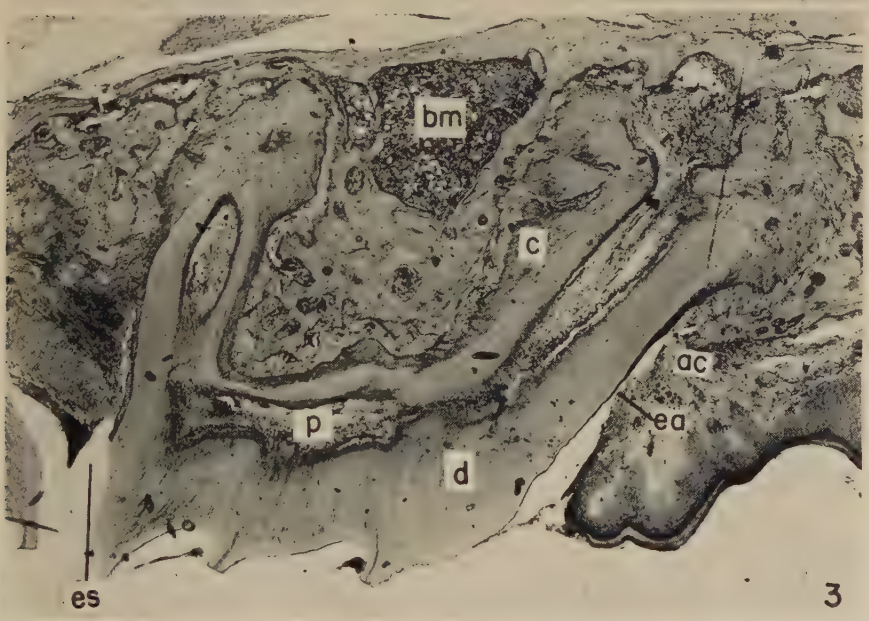
bm
c
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PLATE 2

EXPLANATION OF FIGURES

4 Photomicrograph of the interradicular septum of a mesio-distal section of the maxillary first molar of control mouse, EB 125, 90 days of age. Hematoxylin-eosin stain (× 77). Note normal medullary cavity (mc), dentin (d), pulp (p), periodontal membrane (PDM), and cementum (c).

5 Photomicrograph of the interradicular septum of a mesio-distal section of the maxillary first molar of a mouse, EB 103, 44 days of age, treated with α-estradiol benzoate — dose 1 mg in one week. Hematoxylin-eosin stain (× 77). Note new bone deposited in medullary cavity (nb), dentin (d), pulp (p), periodontal membrane (PDM).

mc
c
PDM
p
d
4

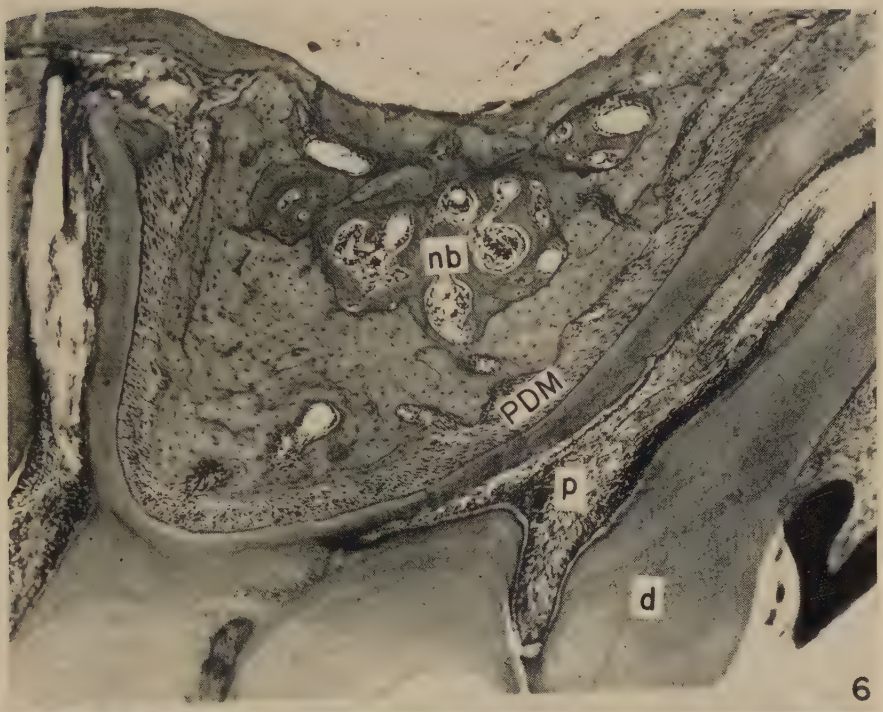
nb
PDM
p
d
5

PLATE 3

EXPLANATION OF FIGURES

6 Photomicrograph of the interradicular septum of a mesio-distal section, maxillary first molar of a mouse, EB 111, 50 days of age, treated with α-estradiol benzoate — dose 2 mg in two weeks. Hematoxylin-eosin stain (× 77). Note new bone deposited in medullary cavity (nb), dentin (d), pulp (p), periodontal membrane (PDM).

7 Photomicrograph of the interradicular septum of a mesio-distal section, maxillary first molar of a mouse, EB 131, 90 days of age, treated with α-estradiol benzoate — dose 7.5 mg in 8 weeks. Hematoxylin-eosin stain (× 77). Note new bone deposited in medullary cavity (nb), dentin (d), periodontal membrane (PDM).


nb
PDM
p
d
6

nb
PDM
d
7

THE CORONARY VESSELS OF THE DOG DEMONSTRATED BY COLORED PLASTIC (VINYL ACETATE) INJECTIONS AND CORROSION

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THREE FIGURES

Even before the time of Servetus and John Hunter observations were made on the coronaries by an Arab physician of the 13th Century, Ibn Nafis, dean of the Mansoury Hospital in Cairo, Egypt. According to Haddad and Khairallah ('36) he stated that the blood is aerated in the lungs and that the heart is nourished by its own vessels. Whitten published a review and analysis of the many methods used prior to 1928. As he pointed out, actual dissection of the vessels is tedious and the finer branches are frequently destroyed. The cleared preparations do not permit easy examination of the deeper vessels. Injection followed by plane roentgenography involves cutting along or across branches thereby destroying their relationship and also fails to give depth. The use of steroscopic x-ray pictures results in overlapping of the branches. Whitten concluded that of the methods reviewed the injection and corrosion methods were the best and recommended celloidin for injecting the vessels. The actual injection requires several days and the casting of the heart cavities is done by an additional procedure.

Since 1928 the injection-corrosion and injection-roentgenography methods have been developed further. Schlesinger ('38) used the injection-plane roentgenography method. His

method for cutting and unrolling the heart was designed to cause the minimal disturbance of the larger branches. Due to the great variation in the anatomy of the coronaries, even in this method, important branches are often sacrificed. Furthermore, it only gives a two dimensional picture, rather than a cast of the actual vessel. An important advance in the injection-corrosion method was the use of "Neoprene" latex by Smith and Henry ('45). Robinson ('49), discusses (p. 342) some of the deficiencies of Neoprene injections, especially their failure to inject many of the smaller vessels.

We believe that colored vinyl acetate preparations are cheaper, simpler and require less time to make. By using high pressure even the arterioles can be injected. For example, using a pressure of 400 to 450 mm of Hg for injecting the heart and lungs of a dog, arterioles in the lung 30 to 40 μ in diameter are filled with the plastic. This method gives relatively permanent colored casts, of both the heart cavities and the blood vessels in their normal anatomical position. Vinyl acetate was previously used by Reynolds ('47) and Delson, Lubin and Reynolds ('48) for the demonstration of the spiral arteries of the rabbit and human ovary.

We have found the papers on the dog coronaries by Meek, Keenan and Thiesen ('29), who used the injection-clearing method, by Moore ('30) and by Pianetto ('39), who used injection-roentgenography, especially valuable. We were unable to find any studies reported on the dog coronaries following injection and corrosion.

TECHNIQUE

Dogs were anesthetized by injecting pentobarbital sodium "Nembutal" (0.032 gm per kilogram body weight) intravenously. Clotting was prevented by injecting heparin (0.5 mg per kilogram body weight) intravenously. The animals were sacrificed by cutting the carotid artery 15 to 20 minutes after injecting the heparin. The heart and lungs were removed to-

gether. Ligatures were placed around the superior vena cava and the blood vessels at the hilum of the lung. This procedure allowed only the aorta and the inferior vena cava to remain patent. The heart was then flushed with tap water to remove any remaining blood. A ligature was next placed around the base of the pericardial sac after which the sac was removed.

The material injected was vinyl acetate (Ward's Natural Science Estab., Rochester, N. Y.). It was sent from the company with a concentration of 12% in acetone. On reaching our laboratory it had a viscosity of 13.8 (relative to water).

The apparatus used for measuring pressure was similar to that described by Whitten ('28, fig. 1).

The inferior vena cava was cannulated and blue or yellow plastic was injected under a pressure of 150 to 200 mm of Hg. The vein was injected first in order to avoid rupturing the interatrial septum. Likewise the aorta was injected with red plastic under a pressure of 200 to 250 mm. This method injects all the cardiac veins and the right heart by way of the inferior vena cava and the coronary arteries and left heart through the aorta.

The injected specimen was allowed to harden for one hour after which it was digested in an aqueous solution containing 2.5% pepsin and 4.0% HCl. The dog heart was completely digested in from 5 to 7 days incubation at 34 to 38°C. A fine jet of tap water was used daily to wash the digested material from the preparation, beginning with the third day of digestion.

A total of 29 hearts was prepared by the above method. The dogs used were of different sexes, breeds, ages and their weights varied from 2.4 to 9.2 kg.

The length and diameter of the blood vessels were found to vary according to the size of the heart and the weight of the dog, however, no exact correlations were made. The measurements included in this paper are intended to give an idea of the relative sizes.

OBSERVATIONS AND DISCUSSION

The coronary arteries

I. The left coronary artery originates in all cases from the aorta as a stem, varying in length from 2 to 8 mm (av. 5.2).

Fig. 1 Posterior view demonstrating.

(1) right atrium; (2) left atrium; (3) left middle atrial artery; (4) left posterior atrial artery; (5) coronary sinus and circumflex artery; (6) left anterior ventricular artery and vein; (7) left marginal artery and veins; (8) left posterior ventricular artery and veins; (9) posterior descending artery and posterior interventricular veins; (10) right posterior ventricular artery; (11) posterior septal artery.

The left circumflex and anterior descending arteries originate from the terminal part of the coronary stem. In addition the anterior septal branch comes off the terminus of the stem in 52% of the specimens. The angular branch originates with the other three arteries in 17% and an atrial branch comes off the stem with the other 4 branches in a single specimen.

Pianetto states that a fossa in the wall of the aorta gives origin to the circumflex and the anterior descending arteries separately. We believe that the length of the stem described above with its two to 5 branches should be called a definite trunk and not a fossa. Moore also considers it to be a stem in the dog. Except for length, this stem is comparable to the trunk of the left coronary artery in man.

A. The left circumflex artery (fig. 1) courses along the left atrioventricular groove, covered at first by the left auricula. Near the posterior interventricular sulcus it turns downwards and follows this sulcus as the posterior descending artery. In 50% of the cases it reaches the apex; in 36% it fails to reach the apex and in 14% it passes beyond the apex along the anterior interventricular sulcus to a maximum distance of 14 mm. The length of the left circumflex (including the posterior descending) varies from 52 to 112 mm (av. 83.1). Its diameter near the stem varies from 1 to 3 mm (av. 1.8). The course, termination, width and length of this artery in our dogs agree in general with the findings of Pianetto.

The left circumflex gives rise to atrial and ventricular branches:

1. Atrial branches: 2 to 5 (av. 3). The anterior branch is the most constant (90%) and longest. It originates from the proximal part of the circumflex artery under cover of the left auricula. It passes upward and to the right, first between the atrium and the pulmonary trunk, then between the latter and the ascending aorta.

The left middle artrial branch, which is found less frequently, divides near its origin into anterior and posterior rami which then ramify over the lateral surface of the left atrium. The less frequent left posterior atrial branch is

usually short and supplies the posterior part of the left atrium. There may be an accessory anterior branch, an accessory middle, or both. An accessory posterior branch is rarely found. Meek et al. reported a short left anterior atrial when there was an accessory anterior atrial branch on the right side. They found the left anterior atrial distributed chiefly to the right auricle, ending near the superior vena cava, while we found it passing more to the left side and having a more extensive distribution. Our middle (intermediate) and the posterior atrial branches were essentially similar to those of Meek et al.

2. The ventricular branches vary from 4 to 11 (av. 5). The left marginal, the posterior descending and the posterior septal are found constantly. The right posterior ventricular is found in 82%, the left posterior ventricular in 76% and the left anterior ventricular in 21%. Additional branches are small and of irregular occurrence.

(a) The left marginal branch runs along the left border of the heart supplying most of the lateral ventricular surface. The number and size of its branches vary greatly depending on the absence or presence of the anterior and posterior ventricular branch. The marginal artery varies in length from 23 to 48 mm (av. 35.0).

(b) The posterior descending varies in length from 18 to 56 mm (av. 35.3). It gives left, right and septal branches. The left branches, 2 to 9 (av. 4), supply part of the posterior surface of the left ventricle. The right branches, 3 to 9 (av. 5), supply a strip of the posterior surface of the right ventricle 9 to 24 mm wide (av. 15.6). The septal branches, 2 to 12 (av. 7), are perpendicular to the posterior descending and supply the posterior part of the interventricular septum.

(c) The posterior septal is a large constant branch which originates from the right posterior ventricular artery in 71% from the posterior descending in 25% and in the remaining 4% directly from the circumflex. It penetrates the heart wall at the crux and immediately divides into several branches; the smaller ones enter the interventricular septum while the

larger go to the atrial septum, reaching in some cases, the anterior surface of the atrium.

(d) The right posterior ventricular arises from the distal part of the circumflex artery and goes primarily to the posterior surface of the right ventricle.

(e) The left posterior ventricular is usually given off the circumflex at a point midway between the marginal and posterior descending. Its length varies from 20 to 45 mm (av. 33.0). In 63% of the hearts in which this branch is encountered it is the only major branch besides the marginal and the posterior descending. It is accompanied by an anterior ventricular artery in 23% and in the remaining 14% a second left posterior ventricular is present.

(f) The left anterior ventricular originates from the proximal part of the circumflex and runs along the anterior surface of the left ventricle. It varies in length from 19 to 29 mm (av. 23.0). In 17% of the hearts in which it is found no other major branches are present except the marginal and the posterior descending. In the remaining 83% it is accompanied by the above two and the left posterior ventricular.

The marginal and posterior descending arteries in our dogs are essentially similar to those described by Moore and Pianetto. Our findings on the right posterior ventricular artery agree with those of Pianetto. No descriptions are given of the left anterior ventricular and the left posterior ventricular in the references cited. Pianetto referred to a branch from the circumflex to the fibrous part of the septum, but failed to identify it in his material. The distribution of the posterior septal in our material corresponds to that of the artery referred to by Pianetto. Because of its constant presence, its relatively large size and its similarity to the septal branch described by Pianetto on the anterior side, we are calling it the posterior septal branch.

B. The anterior descending artery (fig. 2), soon after originating from the stem of the left coronary, runs vertically downward along the anterior interventricular groove. In 55% it terminates at the apex of the heart, in 7% it fails to reach

the apex and in 38% extends beyond it. The shortest artery terminates in 17 mm before the apex and the longest extends 21 mm beyond. The average point of termination for all is 4 mm beyond the apex. The diameter of this artery, near its origin, varies from 1 to 3 mm (av. 1.6) and its length ranges from 45 to 81 mm (av. 67.0). In 7% its lower third deviates

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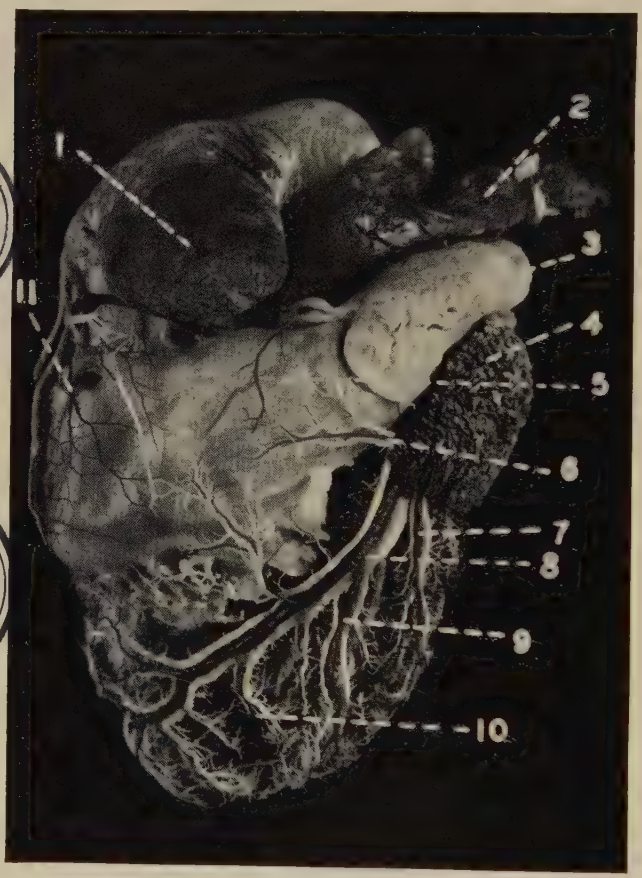

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Fig. 2 Anterior view illustrating.

(1) right atrium; (2) aorta; (3) pulmonary artery; (4) left atrium; (5) right conal artery; (6) left conal artery and veins; (7) angular artery and veins; (8) anterior descending artery and anterior interventricular veins; (9) intermediate artery and veins; (10) apical artery and veins; (11) right marginal artery.

to the left and continues over the anterior surface of the left ventricle, taking the usual place of the apical branch. In this condition one of its right branches passes towards the apex along the interventricular sulcus.

The anterior descending artery has left, right and septal branches.

1. Right branches, 4 to 11 (av. 6), supply an adjacent strip of the right ventricle ranging from 10 to 29 mm (av. 16.0) in width. They are all minor branches, except the first, which supplies the conus of the pulmonary artery and anastomoses with the conus branch of the right coronary artery.

2. Left branches, 4 to 16 (av. 7). Because of their size and importance we are naming the 4 more constant ones from above downwards: angular, intermediate, apical and inferior.

(a) The angular branch passes diagonally across the anterior surface of the left ventricle between the circumflex and the anterior descending. It is constantly found but its place of origin is variable. In 55% it originates from the anterior descending, in 21% from the circumflex and in 14% directly from the stem of the left coronary artery. In 10% there are two angular arteries, one from the circumflex and one from the anterior descending.

We are calling this the angular branch because of the shape of the area supplied. Moore does not give this artery a name but describes it as originating from the circumflex in 75% and from the anterior descending in 25%. In specimens where it originated from the anterior descending (87%) Pianetto called it the *ramus ventriculi sinistri primus* and in 13%, where it came from what he called the left coronary fossa, he referred to it as the *arteria ventriculi sinistri prima aut diagonalis.*

(b) The apical branch passes to the left of the septum and supplies the anterior surface of the apical area, occasionally reaching the posterior surface of the apex. Its point of origin is 14 to 50 mm (av. 29.0) below that of the anterior descending. Some earlier writers, according to Pianetto, described a large branch originating at the junction of the middle and lower parts of the anterior descending, and passing to the left as

the "division of the anterior descending," but they did not name it.

(c) The intermediate branch originates from 1 to 28 mm before the point of origin of the apical branch. A single intermediate branch is found in 41% and two intermediates occur in 17% of the hearts. It supplies the area of the anterior surface of the left ventricle lying between that supplied by the angular and the apical branches.

(d) The inferior branch originates at a distance of 6 to 21 mm below the origin of the apical, and supplies the anterior and inferior surface of the left ventricle. It is found in only 21% of the hearts.

3. Septal branches, 5 to 13 (av. 10). All except the first are superficial perpendicular branches supplying the anterior part of the interventricular septum. We propose to call the first branch the anterior septal for it corresponds to the posterior septal described above.

(a) The anterior septal is found in all specimens. In 55% it originates from the uppermost part of the anterior descending and in the remaining 45% directly from the stem of the left coronary artery. It penetrates deeply supplying all the central part of the septum terminating near the apex. Although various branches from the anterior septal ramify throughout the septum no definite pattern of arrangement was noted such as that reported by Moore and by Pianetto. In 20% a large accessory branch originates from the anterior descending and passes deep into the septum. In contrast to the posterior septal artery, which primarily supplies the interatrial septum, the anterior septal distributes exclusively to the interventricular septum.

II. The right coronary artery (fig. 3) originates from the right anterolateral part of the aorta behind the corresponding cusp. It passes to the right and laterally along the atrioventricular groove, buried in the fatty tissue prevalent in that area. Its first part is covered by the right auricula. It terminates at the posterior interventricular sulcus or shortly (0 to 19 mm, av. 6.0) before it. Near its origin, its width varies

from 1 to 2 mm (av. 1.4) and its length from 29 to 68 mm (av. 52.0). Rarely does this artery, or its branches, extend as far as the borders of the right ventricle and in no case beyond them.

An accessory right coronary artery, varying in length from 5 to 38 mm (av. 19.5), originates from the aorta immediately

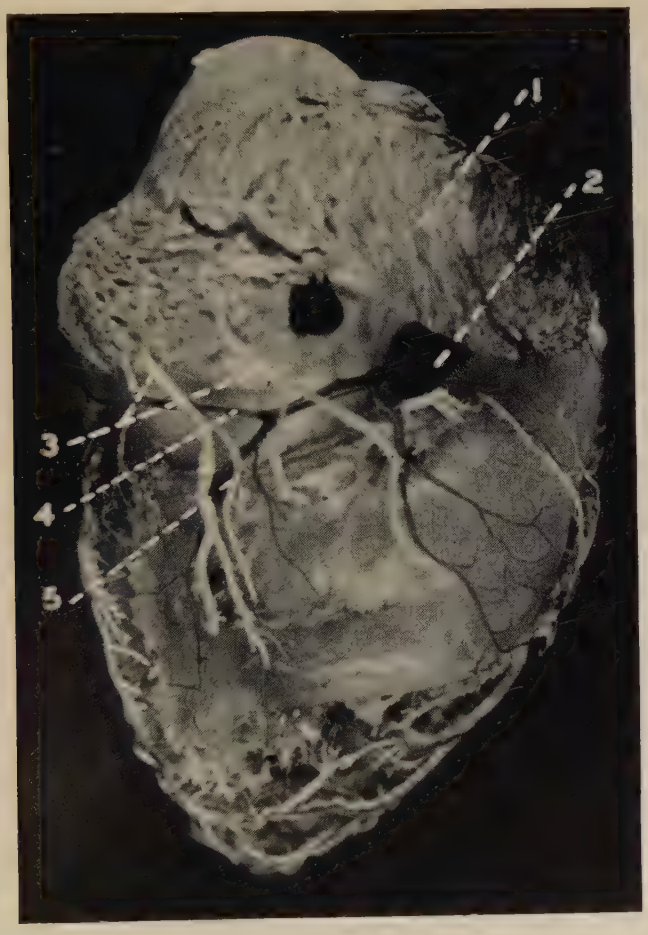

Fig. 3 Right lateral view showing.

(1) Right atrium; (2) right atrio-ventricular groove; (3) independent veins of the right heart; (4) right coronary artery; (5) right marginal artery and independent vein.

beside the right coronary in 81% of the hearts. A second accessory is found in 18%. The accessory, or accessories, together with the first right branch of the anterior descending encircle the conus of the pulmonary trunk. When the accessory is lacking the first ventricular branch of the right coronary takes its place.

The branches of the right coronary are the atrial, ventricular and adiposal.

A. The atrial branches, 2 to 5 (av. 3), are anterior, middle and posterior in position and correspond to similar branches on the opposite side, except that the posterior atrial is the longest rather than the anterior. The right posterior atrial is fairly constant (96%). It originates from the right coronary shortly before its termination, passes upward supplying the posterior surface of the right atrium, lying first between the superior and inferior vena cava, then passing for a considerable distance along the posterior surface of the former vein.

The posterior atrial artery in our specimens corresponds to that of Moore and Pianetto and to the right intermediate atrial described by Meek et al. Judging from its location, we agree with Pianetto that it supplies the sino-atrial node.

The anterior and middle atrial branches originate at various sites along the anterior and middle parts of the right coronary artery. They supply the appendage and lateral surface of the right atrium respectively.

B. The ventricular branches, 4 to 11 (av. 7) compare in size to the short or medium ventricular branches of the left side, except for the right marginal. They originate at varying points along the course of the right coronary, pass vertically downward over the wall of the right ventricle and supply its central part.

1. The right marginal branch is readily identified. Originating from the middle part of the right coronary it passes downwards over the lateral surface of the right ventricle. Its length varies from 17 to 38 mm (av. 30.0).

Pianetto says that earlier investigators described a *ramus marginis acuti,* but that he was unable to identify a similar

branch. Moore calls all of these ventricular branches, without referring specifically to a marginal branch. Because of its constant occurrence, size and location we consider this branch comparable to the marginal branch of the left side.

C. The adiposal are small branches originating from the proximal part of the right coronary and supplying the overlying fatty tissue. Pianetto refers to adiposal branches but he failed to observe them.

The cardiac veins

Due to the recent extensive work on the experimental shunting of arterial blood into the coronary sinus of the dog we are describing the venous system in some detail. Since neither Moore nor Pianetto described the cardiac veins, we are using for comparison with our material the description of the human cardiac veins by Gross ('21, pp. 93–104). In general we have named the veins after the corresponding arteries.

The cardiac veins fall into three major categories, (1) the veins which drain, directly or indirectly, into the coronary sinus (*venae magnae cordis* of Gross), (2) veins which open independently into the right atrium (*venae parvae cordis* of Gross), (3) the Thebesian veins (*venae minimae Thebesii* of Gross).

I. Coronary sinus (fig. 1). The anatomy of the coronary sinus in the dog is similar to that of man. Lying in the left posterior atrioventricular furrow it is limited by two valves similar to the Vieussenian and the Thebesian valve described in man. Sharp constrictions of the sinus are seen in our preparations at the sites of the valves. The sinus opens into the left postero-inferior angle of the right atrium, below the inferior vena cava. In order proximally the following veins open into the sinus; left cardiac, marginal, posterior ventricular, posterior interventricular and right cardiac.

A. The left cardiac vein (great cardiac) is a direct continuation of the sinus, but its caliber is only about one-third

that of the sinus. It continues anteriorly along the left atrioventricular groove. Distally it is continuous with the anterior interventricular while proximally the anterior ventricular opens into it. The angular and atrial branches do not open into it constantly.

1. Left anterior ventricular branches are present only in 21% together with the corresponding artery. Like most of the veins of the dog hearts they are paired and are situated on both sides of the artery.

2. Atrial veins (1 to 2) drain the parts supplied by the left anterior and middle atrial arteries and empty into the left cardiac vein.

3. Angular. In those cases where the angular artery originates from the circumflex the paired angular veins empty into the left cardiac vein. In the remaining cases they join the proximal part of the anterior interventricular vein.

4. Anterior interventricular (fig. 2). Starting as a pair of veins running along the anterior descending artery, they unite at their proximal ends and continue as the left cardiac vein.

There are right and left branches going to the vein of the corresponding side.

(a) Right branches. The first of these, the conal branch, forms an anastomosis with the corresponding branch of the right side. The remaining branches correspond to the right branches of the anterior descending artery and drain the anterior part of the right ventricle.

(b) Left branches. From above downwards paired veins accompany the angular, intermediate, apical and inferior branches of the anterior descending artery draining the areas supplied by these arteries.

B. Marginal. Large paired marginal veins accompany the marginal artery and unite to form a short stem which enters the coronary sinus at or near its distal extremity.

C. Posterior ventricular paired veins are always present in the hearts (76%) which have a posterior ventricular artery. They open into the middle part of the coronary sinus.

D. Posterior interventricular paired veins accompany the posterior descending artery and empty into the coronary sinus near its proximal valve. Right and left tributaries are similar in number and distribution to the branches of the artery. Unlike those in man, the right branches in the dog do not have a definite horizontal pattern.

E. The right cardiac is a rather short unpaired vein running along the right atrio-ventricular furrow posteriorly to join the distal end of the coronary sinus. It drains part of the postero-superior surface of the right ventricle.

F. Atrial branches. In addition to the atrial branches described in connection with the left cardiac vein, one or two posterior atrial branches descend from the posterior atrial surface and enter the middle or proximal part of the coronary sinus.

II. Independent veins of the right heart (fig. 3). There are 3 to 4 veins which begin along the lower part of the outer wall of the right ventricle and ascend vertically to empty separately into the right atrium, immediately above the right atrio-ventricular groove. In addition to these, there are one or two prominent veins which encircle the conus and empty directly into the right atrium between the auricula and the pulmonary artery. These veins collectively drain most of the area supplied by the right coronary artery.

III. Thebesian veins. In our specimens numerous small vertical vessels extend from the veins and communicate with the ventricular cavities. According to our observations these are identical with the Thebesian veins described by Gross and earlier investigators. We failed to demonstrate such vessels over the atrial surface, but this may have been due to the smaller size of the vessels there and to the relatively low pressure used for injecting the right heart.

In general the cardiac veins of the dog do not differ appreciably from those of man. In almost every instance the arteries, even down to their smallest branches, are accompanied by veins which are paired.

There are frequent anastomoses between the ventricular veins of the right and left heart.

SUMMARY

1. Twenty-nine dog hearts were injected by vinyl acetate and corroded with pepsin and hydrochloric acid.

2. The left coronary artery was predominant and originated from a stem of the aorta. It supplied all of the left heart plus one-third to one-half of the right heart.

Left anterior, left posterior ventricular and posterior septal branches originating from the left circumflex artery and from above downward angular, intermediate, apical and inferior branches originating from the anterior descending artery were named and described.

3. A right marginal branch was observed originating from the right coronary artery.

4. The more important cardiac veins were described. Paired veins were found accompanying nearly all the arteries and were given names corresponding to arteries. There was a rich anastomosis between the veins.

5. Veins draining the right heart and emptying independently into the right atrium were observed.

6. Numerous Thebesian veins were observed on the ventricular side in these plastic preparations.

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AN ANALYSIS OF THE VARIATIONS IN POSITION OF THE MENTAL FORAMEN

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TWO FIGURES

INTRODUCTION

Cunningham ('43), Morris ('42), Piersol ('30), and Sicher ('49) state that the mental foramen is located between the apices of the lower bicuspid teeth. Gray ('48) gives this relationship as below the second bicuspid tooth. In view of this discrepancy among the standard anatomy texts as well as repeated failures to locate the mental foramen in injection procedures, a study was made of the relative positions of this opening.

MATERIALS AND METHODS

One hundred unselected, unsexed, adult, dentulous mandibles were studied. This investigation was divided into two phases: (a) to determine the relation of the mental foramen to the lower bicuspid teeth and (b) to determine the relation of the mental foramen to the body and ramus of the mandible. Its relation to the lower teeth was determined by examination. The relation of the foramen to the body and ramus was determined by the use of a plane parallel to the basal or horzontal plane. Morant ('36) suggested a series of standard measurements of the mandible. Among these he listed the standard horizontal plane as follows:

"If a mandible be placed on a horizontal surface with the teeth uppermost, it will normally rest in a unique position, but occasionally it will be found capable of a small rocking displacement. The plane of reference is defined to be that horizontal plane with which contact

is made at three or more points when a vertical pressure is applied at the second molar or its cavity. This is known as the standard horizontal or basal plane, and when placed vertically downwards on the second left molar, the mandible is said to be in the standard horizontal position."

A plane parallel to this standard horizontal plane was selected (fig. 1) by using the height of the most anterior portion of the anterior border of the mental foramen. The midline at the symphysis menti was obtained by anatomic appreciation and the height from the standard horizontal plane

PLANES OF MEASUREMENT

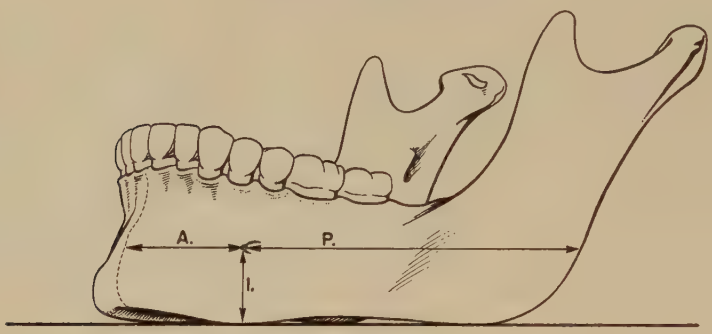

Figure 1

A. Distance between mental foramen and symphysis menti.

P. Distance between mental foramen and posterior border of ramus of mandible.

I. Distance between mental foramen and inferior border of body of mandible.

to the foramen was indicated at the mid-line and at the point where this plane crossed the posterior border of the ramus. Caliper measurements were then taken of the chord distance from the foramen to the inferior border of the mandible (I); from the point at which the selected plane crossed the mid-line anteriorly to the most anterior portion of the anterior border of the foramen (A); and the distance from the most anterior portion of the anterior border of the mental foramen to the point on the posterior border on the ramus at the level of the selected horizontal plane (P).

OBSERVATIONS

The locations of the mental foramina related to the lower teeth are expressed as percentages in 6 categories as follows (fig. 2): (1) foramen anterior to the lower first bicuspid tooth: right, none, left, none; (2) foramen at the apex of the lower

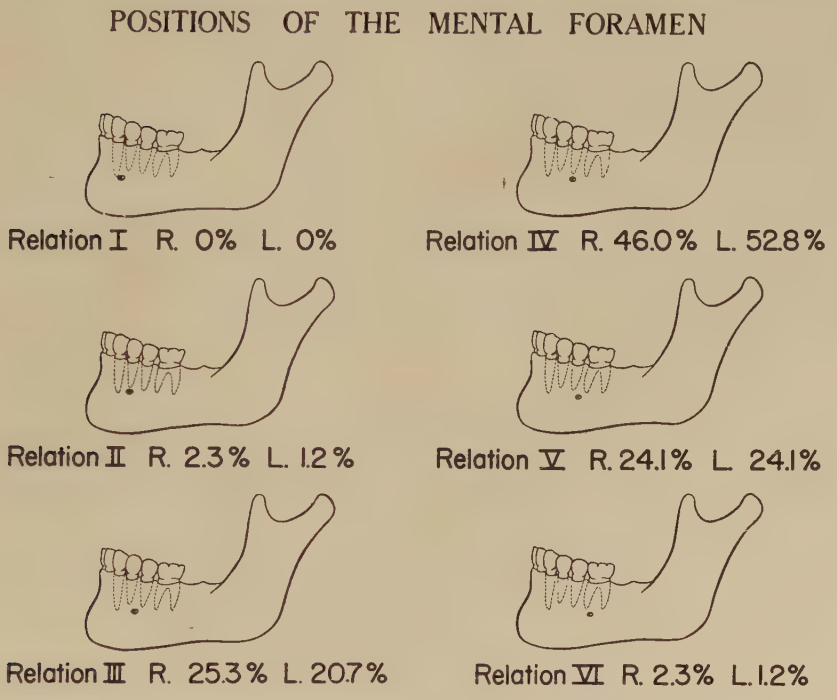
POSITIONS OF THE MENTAL FORAMEN
Relation I R. 0% L. 0%
Relation IV R. 46.0% L. 52.8%
Relation II R. 2.3% L. 1.2%
Relation V R. 24.1% L. 24.1%
Relation III R. 25.3% L. 20.7%
Relation VI R. 2.3% L. 1.2%

Fig. 2 Relation of the mental foramina to the lower teeth expressed in percentages as found in 87 mandibles.

first bicuspid tooth: right, 2.3%, left, 1.2%; (3) foramen between the apices of the lower bicuspid teeth: right, 25.3%, left, 20.7%; (4) foramen at the apex of the second bicuspid tooth: right, 46.0%, left, 52.8%; (5) foramen posterior to the second bicuspid tooth: right, 24.1%, left, 24.1%; and (6) foramen below the root apices of the first molar: right, 2.3%, left, 1.2%.

The measurements taken along the selected horizontal plane are summarized in table 1.

TABLE 1

Relative positions of mental foramina

	RIGHT SIDE	LEFT SIDE
	mm	*mm*
Maximum A	33.2	35.0
Mean A	26.8	26.6
Minimum A	22.2	22.0
Standard Deviation of A	2.33	2.32
Maximum P	95.6	96.2
Mean P	74.6	74.9
Minimum P	59.8	60.5
Standard Deviation of P	6.09	5.90
Maximum I	17.9	18.5
Mean I	15.2	15.0
Minimum I	11.5	9.0
Standard Deviation of I	1.54	1.57

A. The chord distance from the symphysis menti to the most anterior point of the anterior border of the mental foramen.

P. The chord distance from the most anterior point of the anterior border to the point where the selected horizontal plane crosses the posterior border of the ramus.

I. The chord distance from the most anterior point on the anterior border of the mental foramen to the inferior border of the mandible. This measurement taken perpendicular to the selected horizontal plane.

DISCUSSION

Examination of the percentages obtained from the study of the relationship between the mental foramen and the lower teeth reveals that the most common relationship is at the apex of the lower second bicuspid tooth. The usually described relationship (between the apices of lower bicuspid teeth) held in only about one-fourth of the mandibles studied. The possibility of the mental foramen occurring as far posteriorly as the apices of the first molar tooth is of clinical importance since the foramen in this area may be incorrectly interpreted on the radiograph as a pathosis.

Calculations based on the measurements obtained along the selected horizontal plane reveal no correlation between combinations of measurements except P to A + P. This ratio has a value of 0.735 on the right and 0.738 on the left side. This indicates that, along the plane selected, the mental foramen lies approximately one-fourth of the distance from the symphysis menti to the posterior border of the ramus. The technic of anesthetic injections about this area is usually based on the relative location of the foramen to the lower bicuspid teeth. In light of our observations, however, which are at variance with the usually accepted position, it is suggested that this ratio of P to A + P would be a more reliable method of determining the position of the mental foramen for clinical use. The vertical height of foramen from the inferior border cannot be so accurately calculated due to the poor correlation of I to A + P, but the mean distance would be accurate enough for most injection procedures.

CONCLUSIONS

1. The usually accepted description (between the apices of the lower bicuspid teeth) of the relative position of the mental foramina to the lower teeth held in only 20.7 to 25.3% of the mandibles studied. We found the foramen at the apex of the lower second bicuspid (46.0–52.8%) to be the most common position. This is in agreement with Gray ('48).

2. A remarkable bilateral symmetry was demonstrated between the sides of the mandibles studied.

3. The mental foramen was found to lie approximately one-fourth of the distance from the symphysis menti to the posterior border of the ramus. This relationship is suggested as a more convenient method of locating the foramen in clinical practice.

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A REPORT ON THE OCCURRENCE OF A MEDIAN EYE IN A PARTIALLY DICEPHALIC CAT

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TWO FIGURES

INTRODUCTION

The abnormal appearance of a median eye in a vertebrate usually represents the phenomenon called *Cyclopia media.* Such an eye is formed by the fusion of two normally lateral eyes, the left eye representing the left portion; the right eye, the right portion of the cyclopian structure. The situation in a case of an incompletely dicephalic condition is different. Here the right eye of the left head represents the left portion; and the left eye of the right head the right portion of the composite median eye. Two such cases in the pig have been recorded in the literature by Bishop ('14) and Thuringer ('19). The former mentions the presence of two lenses, otherwise only the external features are described. Thus the appearance of one kitten born with two faces, among an otherwise normal litter of 6, seemed worthy of study from an anatomical standpoint.

PROCEDURE

The abnormal kitten lived for 24 hours after birth. It was received in the laboratory after an interval of two days following its death, and fixed in 10% formalin. Photographs (fig. 1) and roentgenograms were taken. The head was removed, stained in bulk with Grenacher's Borax Carmine, imbedded in celloidin and sectioned.

OBSERVATIONS

Besides the three eyes, the kitten had two noses, two mouth openings, but only two normally placed ears. In the skull there was a double set of nasal bones, frontals, premaxillaries, maxillaries, and mandibles. The two mouth cavities coalesced posteriorly, and the two tongues were fused at their bases.

The median composite eye was of approximately normal size, but almost completely hidden under the hairs of the surrounding skin. Externally it appeared like a normal eye except that the lower eyelid was double, the two parts meeting at an angle of about 45°. The features of the internal anatomy may be observed in the section reproduced in figure 2.

The orbit was single, also the cornea and the aqueous chamber. The sclerotic coat was approximately spherical, but arising from the inner surface of the posterior wall a median septum was formed from this sclera. It separated posteriorly the components of the double eye. There were two separate choroids each of which formed a completely enclosed bulb around their respective vitreous chambers. The retinae were rather distorted in the sections, but in part clearly visible. They lined the inner surface of the choroids behind the lenses. There was no indication of the presence of an iris or pupil. This also appeared to be the situation in the two lateral eyes as well as in the double median one. Adhering closely to the inside of the front of the double vitreous chambers were two lenses, but there were no suspensory ligaments or ciliary processes. Since these lenses were of normal size, they occupied a relatively very large amount of space in the chambers. There was apparently only one set of eye muscles. However, the individual muscles were difficult to recognize in the sections and it was not clear whether or not the oblique muscles were present. Owing to post-mortem changes the optic nerves could not be traced satisfactorily. The ophthalmic artery divided to form two branches: one to each part of the composite eye.

DISCUSSION

In the absence of evidence from the optic nerves, only the presence of the two lenses remain as an argument for the assumption that the composite median eye was formed by the coalesence of two separate optic vesicles each of which induced the formation of lenses.

SUMMARY

A kitten, *Felis domestica*, was born with two distinct faces. As a result of this partially dicephalic condition, the right eye of the left face and the left eye of the right face had fused to form a median eye between the two noses. The upper palpebra was single; the lower palpebrae had fused in a V-shape. The cornea and aqueous chamber were single as was the sclerotic coat forming but one eye-ball. Within this, the choroids formed two separate and completely closed balls each of which contained a lens of normal size in a vitreous chamber of greatly reduced size. Ciliary bodies and iris were lacking, and the eye no doubt was not functional. There was but one set of eye muscles, the oblique ones not being clearly discernible. An optic nerve could not be traced but this may be due to the poor state of preservation of the nervous tissues. Opthalmic arteries were leading to each of the two eye halves.

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PLATE 1

EXPLANATION OF FIGURES

1 Frontal view of the partially dicephalic kitten. The median eye is completely hidden by the hairs.

2 Section through the median double eye of the partially dicephalic kitten.

1
2

A LIPASE REACTION IN PHAGOCYTES FROM THE PERITONEUM OF RATS TOWARD PREVIOUSLY STAINED FAT

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THREE FIGURES

INTRODUCTION

The study of phagocytosis by free cells of foreign matter introduced into the cavities of the animal body, usually has been approached from a morphological rather than from a physiological viewpoint. These free cells, which increase greatly in numbers in the presence of foreign bodies, have been studied intensively in many animals. Webb ('31) has described those of the rat, identifying them as mast cells, eosinophils and monocytes; while neutrophils seem to be absent in the peritoneum of this species.

The introduction of histochemical methods, recently reviewed by Glick ('49), has opened a new approach by which the physiological nature of cell activities may be investigated. Fat absorption by cells would seem to be a particularly rewarding process to study by a combination of the traditional histological methods with these newer techniques, involving specific chemical reactions.

Regarding fat absorption by differentiated tissue cells a number of morphological studies are available especially those by Munk (1884) on intestinal epithelia, by Wotton and Zwemer ('39) on intestinal and liver tissues, by Wegelin ('13) on cardiac tissues, by Modell ('33) and Zwemer and Wotton ('44) on kidney tissue, and by Singer and Zwemer ('34) and Zwemer, Wotton and Norkus ('38) on the adrenal cortex cells; by

Hirsch and Weinhouse ('40), Merklen, Waitz and Kabaker ('32), Fiessinger and Marie ('09), Smithburn and Sabin ('32), and Sabin, Smithburn and Thomas ('35) in pathological conditions.

MATERIAL AND METHODS

Twenty-six white rats were used in this study. Before use the animals were kept for several days in the laboratory and were observed to be healthy and normal. Test doses of 0.5–1.0 cm^3 of cod liver oil, saturated with Sudan IV, were injected intraperitoneally. The animals were killed at various time intervals after the injection as follows:

Hours after injection	1	2	3	4	5	6
Number of rats killed	1	3	6	7	5	4

In order to avoid possible artifacts through the action of fat-solvent anaesthetics, the animals were stunned by a blow on the head. The abdomen was slit open and everted in such a way that smears could be made by passing glass slides along the peritoneal surface over the inside of the body walls as well as over the organs. Precaution was taken to make the smears where some fat was seen. These smears were allowed to dry, and then were placed immediately in 10% formalin for further fixation. After a time interval of 10 minutes to several hours, the formalin was removed by successive washings in distilled water. Some of the slides were stained for 45 minutes to several hours (overnight) in a Sudan IV solution, made by dissolving 0.5 gm of Sudan IV in 100 cm^3 of 70% alcohol and an equal volume of acetone. They were afterward rinsed in 50% alcohol, followed by two or three washings in distilled water. From here on all slides were processed similarly whether they had been stained or were left unstained. Following the method of lipase identification by Gomori ('46a and b), as reported by Glick ('49), they were immersed first for 30 minutes in a 1.5% solution of lead nitrate and secondly, after four 5-minute rinses in distilled water, for 5 minutes in a freshly prepared 5% ammonium hydrosulfide solution, followed by three rinses in distilled water. They

were finally, as suggested by Zwemer ('33) and Wotton and Zwemer ('35), mounted in glychrogel for permanent mounts and in glycerine for temporary use.

OBSERVATIONS

The 300 slides were arranged in such a way that there were available for study three series for each animal: (1) Stained with Sudan IV to intensify the stain already present in the injected fat, and further processed by the Gomori method for lipase identification; (2) processed in the same manner without any additional Sudan IV staining; and (3) stained but not further processed and used for comparison.

All observations of these slides revealed a striking cellular response to the injected, previously stained fat. There was no noticeable difference between the reaction of cells from animals of different ages nor between those killed at different time intervals after injection. In all cases phagocytes accumulated about the droplets of injected previously stained fat. Some adhered closely to the surface of a fat globule, others in a still later phase rested like a cap on the top of the fat drop. This surface association appears as a preliminary step to the absorption of fat by the phagocytes. At certain very small but clearly defined areas on the surface of such cells engaged in fat absorption a fine stream of fat could be seen entering the cytoplasm (figs. 2 and 3). These points of entry may be single or multiple in a particular place. In the slides processed for lipase identification whether or not stained with Sudan IV, a dark brown area is conspicuous in the region where the cytoplasm of the phagocyte touches the fat gobule (fig. 1). In some cases black films or lines were found associated with these dark brown areas. Presumably the reactions are brought about by a lipase breaking down the triglycerides of fatty acids producing free fatty acids which combine with lead ions present in the lead nitrate to form lead soaps. The addition of ammonium hydrosulfide

solution results in the formation of lead sulfide indicated by the dark brown areas. The black films or lines are due to an excess of lead ions combining with sulfide ions to form a black precipitate.

In the slides of the control series no reactions indicating lipase activity were apparent.

Two types of cells take part in this absorption of fat and both display the described reactions. The more common type is a mononuclear or histiocyte with lobose pseudopodia; the other, more rarely seen, resembles a fibrocyte with filose pseudopodia. The phagocytic powers of these types of cells are apparently different, the former taking in large quantities of fat, and the latter only a few droplets at a time. Both cell types showed no difference in their ability to approach and cling to fat droplets, in fact it was frequently observed that cells of both types were attached to the same fat globule, and they could be found on the surface of large masses of fat as well as on small droplets.

DISCUSSION

The above observations strongly suggest that two types among the free cells of the peritoneum of the rat are active in the phagocytosis of fat, namely the mononuclears or histiocytes, and to a lesser extent, certain fibrocyte-like cells. Other free cells such as the numerous lymphocytes and the polymorphonuclear leucocytes showed no affinity for the injected fat. This finding supports the views of Clark and Clark ('30) that only macrophages and monocytes participate in the process of phagocytosis in the peritoneal cavity.

The morphological aspect of fat absorption by free cells differs strikingly from that displayed in food ingestion by protozoan Amoebae. There is no evidence of fat droplets being surrounded or overflowed by pseudopodia and included in food vacuoles. The process is similar to that demonstrated by Wotton and Zwemer ('39) for columnar epithelial cells of the small intestine and the parenchymal cells of the liver

where the droplets take on an hour-glass shape as they pass though the cell border into the cytoplasm. When a histiocyte or a fibrocyte attaches itself to the surface of a fat droplet, a rather extensive interface is established. However, only at one point or a few limited and clearly defined areas within this interface does fat absorption actually take place. At these points, a narrow stream of fat is seen entering the cytoplasm (figs. 2 and 3). Within the cytosome, the fat remains as a streak at first (fig. 3).

By applying the Gomori method of lipase identification, it has been possible to demonstrate that the morphological phenomena described above are accompanied by hitherto unsuspected physiological processes in the nature of a digestion of the fat. This is evidenced by the identification of a lipase reaction at the cell-fat interface. The nature of the lipase identification technique is as follows. Wherever a lipase brings about hydrolysis of fat, the fatty acids thus produced react with lead nitrate used in the procedure to form metallic soap which, following the subsequent treatment with ammonium hydrosulfide, yields a precipitate of lead sulfide which, according to Gomori appears "golden brown" at sites of lipase activity. This chemical test relies on the presence of free fatty acids. If our interpretations be correct, the demonstration of such brown areas at cell-fat interfaces proves the presence of free fatty acids in the fat which is in direct contact with the cell that is to ingest it, and the action of a lipase produced from the surface of the cell as part of the process of fat phagocytosis.

SUMMARY

Cod liver oil previously stained with Sudan IV was injected into 26 rats, and the phagocytic action toward this fat of free cells was observed in the peritoneal cavity. Two types of cells only were active. These were histiocytes, and more rarely, fibrocyte-like cells. The former cell type absorbed fat in considerable quantities, the latter only in small droplets.

Ingestion by the cells of the previously stained fat took place at specific points within the extensive interface between cell and fat, and the ingested fat was observed as streaks within the cytosome. By applying the Gomori method lipase identification, it was possible to demonstrate the presence of free fatty acids in the fat near the surface of the cell, which was about to ingest it. This suggests the existence of a lipase reaction at the cell-fat interface indicating the possibility of an extracellular digestion of fat as part of the absorption phenomenon.

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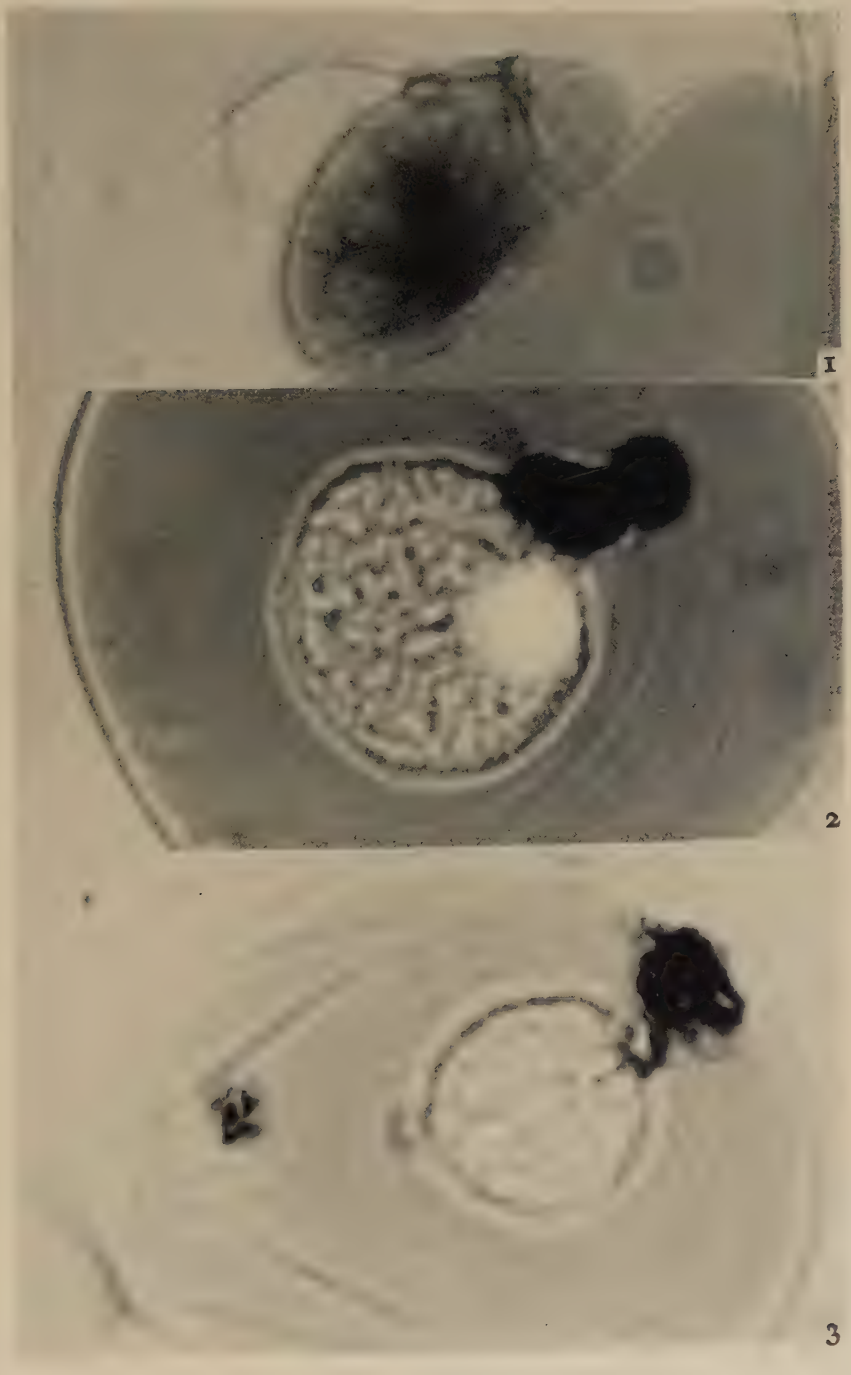
PLATE 1

EXPLANATION OF FIGURES

1 A phagocytic cell adjacent to a large fat drop. The dark area indicates the lipase reaction. Enlargement about × 1300.

2 A round phagocytic cell on top of a fat drop. Note dark area and 4 thin lines of fat entering the cell from this area. (The white area is a flare spot.) Enlargement about × 1300.

3 Similar to figure 2. Note the line of fat entering the cell. Enlargement about × 1300.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REDIFFERENTIATION OF SEBACEOUS GLANDS IN THE MOUSE AFTER TOTAL EXTIRPATION WITH METHYLCHOLANTHRENE[1]

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SIX FIGURES

INTRODUCTION

During some exploratory studies on the effect of methylcholanthrene upon the skin of mice, we have observed the disappearance of sebaceous glands within 4 days after a single application (observed also by Simpson and Cramer, '43, '45). We have further noted that a sebaceous gland grows anew from the external root sheath grown over the former site of the opening of the sebaceous duct. The rapidity of this new glandular differentiation, however, is dependent upon the state of activity of the associated hair follicles. In follicles with actively growing hairs, complete glandular replacement occurs within 10 days of the initial application of the carcinogen. In "resting" or reorganizing follicles the process is much slower. This study presents, in addition, an excellent method for the study of the growth potentials and differentiation of these organs. Further experiments now in progress will be reported in subsequent papers.

MATERIAL AND METHODS

Mice used in these experiments were primarily of the C57 Black strain. Other strains from this laboratory displayed no essential differences as regards the sebaceous

[1] Partially supported by a grant from the United States Public Health Service, R. G. 2125, and by a grant-in-aid from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

gland and hair growth relationship. For controlled stages of the hair growth cycle club hairs of areas in the telogen phase ("resting") were plucked. This, as Chase ('49) has shown, will initiate the growth of the succeeding hair generation in that area alone. Animals of 4 to 6 months of age were chosen in order to avoid the frequent "spontaneous" growth waves of younger mice.

The treatment consisted of one thorough painting with a camel's hair brush of 0.6% methylcholanthrene in benzene. The controls were painted with benzene alone. One series consisted of painting at the 6-day stage of hair growth (6 days after plucking). Tissues were then taken each day thereafter for a period of 13 days. Many of these biopsies were from the same animal and from 5 to 8 biopsies were taken from each animal. Hair development and rate of growth were normal, the characteristic "nearly complete" stage of growth being obtained at the end of that time (17–19 days after plucking). A corresponding series consisted of painting at the "resting" phase (unplucked). Tissues were again taken at each day following for a period of 13 days. In these cases epilation occurred at approximately 5 days, and in some places new follicle activity was apparent before the end of the 13-day period. Another series involved the plucking of 4 different areas on an animal at different times and leaving one specific area unplucked. In this manner when the 5 areas on an animal were painted, one was at 10-day growth stage; one at 6-day; one at 3-day; one at 0-day (plucked then painted at once); and one at 0-day but unplucked. Tissues were taken at 6 and at 8 days following painting. Hair development was normal in all plucked areas, having reached the characteristic post-plucking stage at the time the tissues were taken. In the unplucked area, epilation had just been completed when tissues were taken.

Each biopsy was cut into three pieces. One piece was fixed in formol calcium, postchromated, and imbedded in gelatin. Frozen sections were treated with sudan black or with other oil soluble dyes. Untreated sections were studied under the

phase microscope. Material fixed in Bouin's was stained with Delafield's hematoxylin. Skin fixed in Helly's was stained with eosin-methylene-blue.

RESULTS

After the application of methycholanthrene to the skin of mice, regardless of the activity of the hair follicles (plucked and unplucked), the early changes in the epidermis and in the sebaceous glands are alike.

Twenty-four hours after the application of the carcinogen the epidermis is thicker than in normal skin and the cells are hypertrophied. The cell boundaries are not smooth as in normal epidermis but they show clear intercellular bridges. The sebaceous glands appear fairly normal, except that the lipid droplets are coarse and clumped, unlike those in the glands of normal skin where they are delicate and evenly distributed. Even the few lipid spherules in the immature peripheral sebaceous cells are coarse and clumped. The dermis is sown with stellate macorphages which contain lipid droplets. The cells in the dermis which contain very small pigment granules also contain lipid bodies, but the larger melanophores with large pigment granules are free of demonstrable lipids.

After 48 hours the epidermis has differentiated into 4 to 6 layers in thickness, showing a clear stratum granulosum (cf. Cramer and Stowell, '42). The orifices of the pilosebaceous units are dilated, and the sebaceous ducts are shallow and full of sebum. The sebaceous cells show massive coalescence of their lipid spherules. All of their nuclei are pycnotic or hypochromatic and the cell boundaries are indistinct. By proliferation, the peripheral, non-sebaceous cells of sebaceous glands have pushed the sebaceous cells into the orifices of the pilosebaceous units. The dermis shows an increase in the cell-population, characterized by lipid-containing macrophages and leukocytes.

On the third day there is an increase in the number of hypertrophied cells in the epidermis. Cellular hypertrophy extends

into the outer root sheath of hair follicles to the level of the arrectores pilorum muscles. All of these cells show conspicuous intercellular bridges. Fragments of sebaceous glands rest in the dilated exits of pilosebaceous units. The external root sheath has grown over the original gap of the sebaceous gland duct, forming a smooth wall.

On the 4th day the epidermis shows a stratum granulosum, several layers thick, and a very thick stratum corneum. In the stratum corneum, the upper layers are richly sudanophilic and the lower ones, just above the stratum granulosum, show a delicate sudanophilic stippling in a sudanophobic background. There are no traces of sebaceous glands. Even the pilosebaceous exits contain only traces of sebum. The walls in the upper part of the follicle give no indication that sebaceous glands and ducts had ever been present.

From this stage on, the events in the skin follow two different paths. If the hair follicles, now devoid of sebaceous glands, are actively producing hair, new sebaceous glands will begin to form on the 5th day. If, on the other hand, hair is not growing, then sebaceous glands will not initate regeneration until the growth of the hair is under way.

In adult mice, the cyclic growth of hair consists of a period (anagen) of approximately 17 days for active growth; a period (catagen) of two days for the completion of hair base and club, and a period (telogen) of 10 days to several weeks for the resting phase (Dry, '26). After the application of methylcholanthrene to areas in the resting phase, the club hairs are soon shed. Usually, this epilation starts on the 4th day following treatment and is completed by 6 or 7 days. The follicular sheath then becomes a solid cord of cells many of which are greatly hypertrophied. The complete reorganization of such follicles, before the new hair generation is initated, is variable and may require 10 days or longer. This means that sebaceous glands will not begin to grow in these follicles until 10 days or longer, after the application of methylcholanthrene.

In skin anointed 6 days after the club hairs have been plucked, the follicles already have rapidly growing new hairs and the neogenesis of sebaceous glands begins on the 4th to the 5th day. This, then, occurs not more than two days after the disappearance of the previous glands.

It is necessary to call attention at this time, to minute, perinuclear lipid particles which are found in the cytoplasm of normal and hypertrophied epidermal cells (previously described by Montagna, '49). These lipid bodies are present in the epidermal cells (fig. 1) as well as in the cells of the external root-sheath and in the bulb of growing hairs. On the 4th and 5th days after the application of methylcholanthrene these cytoplasmic lipid bodies are more numerous than elsewhere, in some cells of the external root sheath above the level of the arrectores pilorum muscles.

In some cells (fig. 2) the nucleus is completely encircled by these lipid spherules. These cells are found singly or in small groups, and their size or shape is not different from those of other cells in the external root sheath. This is the earliest observation of sebaceous differentiation from external root sheath cells. Further differentiation of these cells is brought about by continued accretion of lipid droplets in the cytoplasm (figs. 3, 4). Once completely laden the cells begin to enlarge until they enlarge several times their original size (figs. 5, 6). Once these foci of sebaceous differentiation begin, they progress toward the medial border of the external root sheath. As the mature sebaceous cells in the median border of the external root sheath undergo degeneration they leave behind a path which becomes the excretory duct of the new gland. As the differentiating sebaceous cells enlarge they cause focal evaginations of the external root sheath into the surrounding dermis and thus establish the fundi of the new glands.

This local differentiation does not proceed at an equal rate in all of the follicles. After 7 days some occasional follicles are still devoid of sebaceous glands, but from 9 to 13 days after the application of the carcinogen, most of the glands

have fully reformed, and do not differ from those in untreated skin.

DISCUSSION

Simpson and Cramer ('43, '45) have shown that sebaceous glands are destroyed 4 days after one application of methylcholanthrene. They have observed that sebaceous glands may regenerate but that this process may take longer than two weeks. Regeneration of sebaceous glands and hair follicles in scars over burned areas has been described by Lacassagne and Latarjet ('45). The present study concerns itself only with the mechanics of differentiation of sebaceous cells from apparently "indifferent" epithelial cells, and the effect of the carcinogen is interesting only in that it provides a convenient tool in this problem.

Since sebaceous glands differentiate anew from "indifferent" epithelial cells within one or two days after total destruction provided that the follicles contain actively growing hairs, the growth of the glands must be dependent upon that of the hairs. It explains at once why the regeneration of glands in previously plucked skin is much more rapid and predictable than that in unplucked skin.

In these preparations the differentiation of the glands can be followed from the beginning without interference from other sudanophilic elements. It is becoming increasingly evident that the earliest sebaceous differentiation is initiated by an increase in the number of the perinuclear lipid bodies in the epidermal cells. (Montagna, '49, in the sebaceous glands of the cat finds sebaceous differentiation associated with discrete osmiophilic bodies.) Since these early lipid elements resemble the Golgi bodies of Baker ('44, '49), Thomas ('48) and Cain ('49), and since they are normal elements of the cytoplasm of epidermal cells, we are inclined to consider them Golgi elements.

SUMMARY

1. In the skin of mice, sebaceous glands are completely destroyed by methylcholanthrene 4 days after one application.

2. If the follicles contain growing hairs, sebaceous glands begin to differentiate anew from the cells of the external root sheath on the 5th day after application of the carcinogen. The glands are fully reformed by the 10th day. In follicles where the hair bulbs are not yet growing the sebaceous glands will not regenerate until growth of the bulb is under way.

3. Sebaceous differentiation is associated with perinuclear lipid globules which are normally present in all of the epidermal cells. These lipid bodies are identified with the Golgi bodies.

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PLATE 1

EXPLANATION OF FIGURES

All of these figures are of frozen sections from tissues fixed in formol calcium, postchromated, and treated with Sudan black.

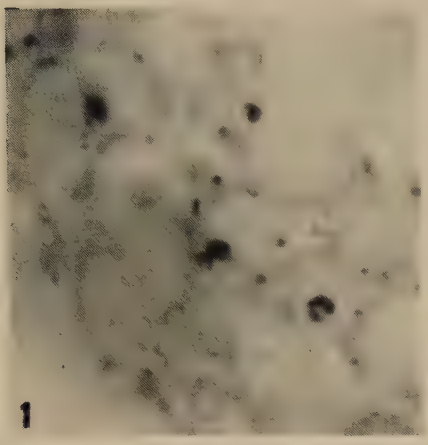
1 Cells in the external root sheath of a mouse three days after the application of methylcholanthrene. Note the distribution of the larger perinuclear sudanophilic bodies. The smaller ones are not resolved clearly. × 1800.

2 Cells showing the first step in sebaceous differentiation. Note the increase in the perinuclar sudanophilic bodies. Four days after methylcholanthrene. × 1125.

3–4 Progressive accumulation of lipids in cytoplasm. As yet there is no appreciable increase in the total dimension of these cells. Four days after methylcholanthrene. × 1125.

5 Nearly mature sebaceous gland showing the progression of sebaceous accumulation from the earliest steps in the lower right-hand corner to mature hypertrophied cells in the upper part. Five days after methylcholanthrene. × 1125.

6 Mature sebaceous gland. Compare with other figures. Seven days after methylcholanthrene. × 1125.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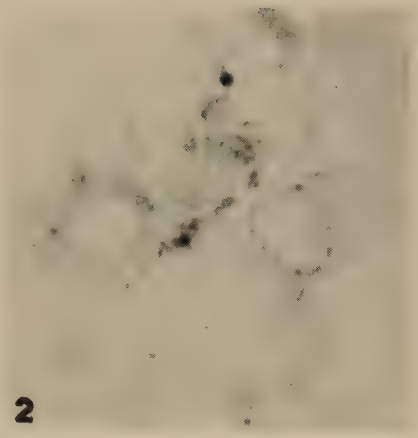
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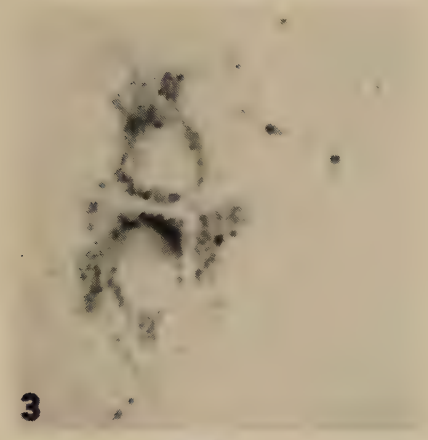
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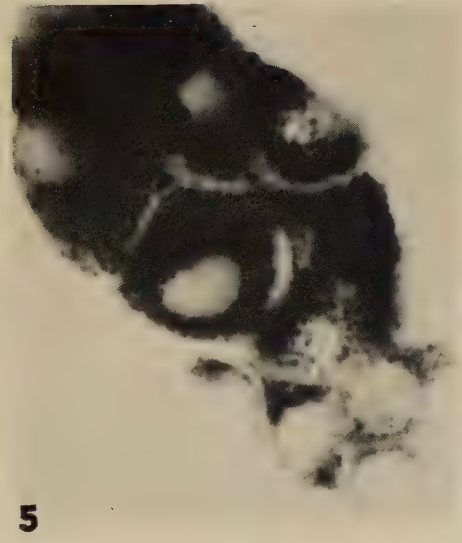
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STUDIES ON THE INGUINAL REGION

III. THE INGUINAL CANAL

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TWO FIGURES

Anatomists and surgeons disagree as to the fundamental anatomy of the inguinal canal despite the fact that the region is frequently dissected in the laboratory and is certainly important to the general surgeon. Many descriptions are a mere listing of the layers and the illustrations so stereotyped that they are of little practical importance. The literature on the gross anatomy of the inguinal canal and related structures is very extensive. The clinical literature is voluminous.

In reference to the rate of descent of the testes and the structure of the gubernaculum the literature is contradictory. Frankl ('00) reported smooth muscle and connective tissue in the gubernaculum. Forssner ('28) could not find smooth muscle and concluded that the whole structure gradually transformed into mucoid tissue. Wyndham ('43) states that the gubernaculum is very vascular and contains both striated and unstriated muscle.

MATERIALS AND METHODS

The material used in this study is of three kinds. During the past 10 years 145 careful dissections have been made of the lower abdominal wall region with special emphasis on the inguinal canal. This has been done in cooperation with medical students. To supplement these dissections observations have also been made on the inguinal canal in already dissected specimens at the University of Pittsburgh and a

few at Northwestern, University of Chicago and Columbia. A total of 290 body halves have been studied. Twenty indirect inguinal hernias have been encountered. The inguino-hypogastric region in 36 fetuses, ranging in age from near 4 months to more than 7 lunar months was also investigated. The gubernaculum and fetal testes were removed from 10 specimens and studied histologically.

RESULTS

This dissection of the inguino-hypogastric region in 36 fetuses ranging between 9 cm and 30 cm (crown-rump) length gave the following results. The processus vaginalis measured 1 mm in fetuses 9 to 12 cm long. At this stage the testes have not yet reached the internal ring. In fetuses 16 to 18 cm long the testes were at or near the orifice of the processus vaginalis, inside the canal in those 22 to 24 cm and present in the scrotum in specimens 25 to 27 cm in length. Some variations were noted. In two fetuses 18.5 cm in length the testes had passed through the internal ring but in one fetus (21 cm long) both testes were proximal to the internal ring. The obliteration of the peritoneal protrusion, except the tunica vaginalis portion, and the closure of the opening in the internal ring occur at unequal rates. When the testes were in the scrotum the peritoneal protrusion was obliterated in some fetuses but still patent in about an equal number. If obliterated it was complete — not even a dimpling of the peritoneum in the internal ring remained.

No muscle tissue of any kind could be demonstrated in 10 histological preparations of fetal gubernaculum using hematoxylin and eosin stains. No evidence could be found to indicate that the gubernaculum extends into the scrotum. It was confined to the processus vaginalis in all instances.

A few examples of *Ectopia testis* were noted. In one fetus the right testis had turned transverse to the entrance of the internal ring. In another fetus both the testis and the gubernaculum were about 4 times the size of the normal testis on the opposite side.

Formation of the inguinal canal. The time and the factors which result in the formation of the inguinal canal are not entirely understood. Wells ('44) says "it represents the inferolateral portion of the coelom which appears grossly to have been pushed out by the gubernaculum testis." Forssner ('28) claims that there is no actual downgrowth of the processus. Estimates on the rate of growth vary greatly also. Wells ('44) reports no evagination before the 6th lunar month, but Frankl ('00) states that the processus is about 1 mm in length at the end of the 5th month.

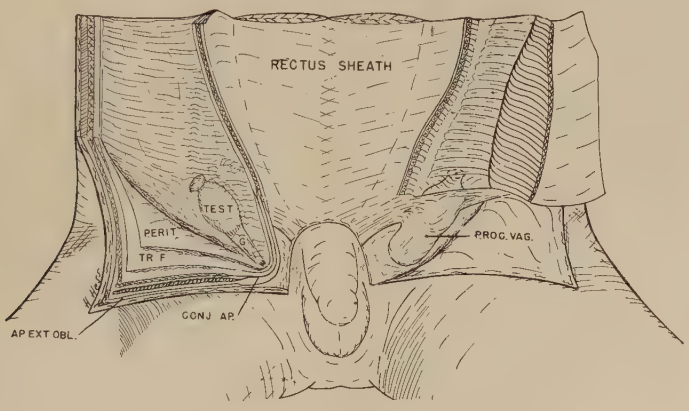

Fig. 1 The processus vaginalis and testis in a fetus 23.5 cm (C.R.) in length. On the right side the processus has been opened to show the layering present. The testis had just entered the internal ring.

The inguinal canal and associated structures in the adult. The inguinal canal in the adult is usually described as an oblique passageway from the internal to the external abdominal rings. At birth the two rings are approximately opposite each other. The external one remains fixed in position but the internal ring in the transversalis fascia is pulled laterally by the growth of the abdominal wall. Thus the obliquity of the canal is acquired as a process of growth. The formation of the inguinal canal does not in any way affect the layering of the ventral abdominal wall.

(a) The peritoneum. A small dimpling was present in several adult specimens, some unilateral and some bilateral. However, these are best seen at operation or autopsy. The shrinkage of tissues in the cadaver usually renders small protrusions inconspicuous.

(b) Subperitoneal tissue. In thin individuals the amount of subperitoneal tissue was negligible and in stout ones it was often considerable.

(c) The transversalis fascia. This is a strong definite layer lying in contact with the inner aspect of the transversus abdominis muscle. Just lateral to the deep inferior epigastric vessels a fascial diverticulum extends into the inguinal canal. This protrusion is the internal spermatic fascia. The mouth of this protrusion is known as the internal "ring" since the margins are held rather firmly to the peritoneum. The transversus fascia can be separated from the peritoneum at every point except at the internal ring. The transveralis fascia can be separated from the transversus muscle upward to the semicircular line. At this point it seems to be incorporated in the dorsal layer of the rectus sheath.

(d) The conjoined aponeurosis. The aponeuroses of the internal oblique and transversus muscles unite to form the conjoined aponeurosis as pointed out in an earlier paper of this series, Chandler and Schadewald ('44). Both the medial and inferior extent of both muscles varies considerably. The transversus seldom extends inferiorly to the inguinal ligament but the hiatus is filled in by aponeurotic or more often by fascial tissue. The internal oblique also varies greatly in its medial extent but its fascia gets an attachment to the whole of the inguinal ligament in almost all instances (Anson, '38). However, its main origin is from the strong iliopsoas fascia. These two muscle layers occasionally present areas of weakness — regions that are fatty or where the muscle tissue is wanting locally. In general these areas are best observed by transillumination.

The middle covering of the spermatic cord is the cremasteric. It is both muscular and fascial. The muscle fascicles

are derived from the internal oblique. The transversus muscle is said to give origin to some of these muscle fasicicles but in this series of cadavers the inferior extent of the transversus abdominis was not to the level of origin of the processus vaginalis. The main attachment of the muscle fascicles was to the iliopsoas fascia and not to the inguinal ligament.

The transversalis fascia is sometimes said to form the medial boundary of the femoral ring. In this region the transversalis fascia is closely adherent to the conjoined aponeurosis, especially as the latter inserts along the pecten of the pubis. Thus it is the strong conjoined aponeurosis that forms the medial margin of the femoral canal. This boundary is 2 to 4 cm lateral to the lacunar ligament.

(e) The external oblique. This layer most nearly fits the usual text book description. Its inferior free edge is the inguinal ligament. Although the fascia lata of the thigh, the innominate or true deep fascia of the abdominal wall and the iliopubic tract all join to the inguinal ligament it is not truly continuous with any one of them. The lateral attachments of the inguinal ligament are the anterior superior spine of the ilium and the strong iliopsoas fascia. The medial end inserts into the pubic tubercle and through the pectineal fascia to the iliopectineal line. Between the attachments to the iliopsoas fascia and the pubic tubercle the inguinal ligament is quite mobile. The portion having an attachment to the pectineal fascia and iliopectineal line is known as the lacunar ligament. The fibers of the lacunar are usually shown passing backward and medially from the inguinal ligament to the pecten of the pubis. McVay and Anson ('40) correctly state that all fibers of the lacunar ligament are parallel to the inguinal ligament.

These authors also question the existence of an iliopubic tract. In well developed specimens it is a stout, well-defined structure. It can be seen best by cutting the inguinal ligament near its mid-point and reflecting each end. It serves as an attachment of the inguinal ligament to the tissues that lie

behind it. A reflected ligament is also described as arising from the inguinal ligament, the lacunar ligament or from the opposite aponeurosis of the external oblique. This is rarely present — it was noted only three times in this study.

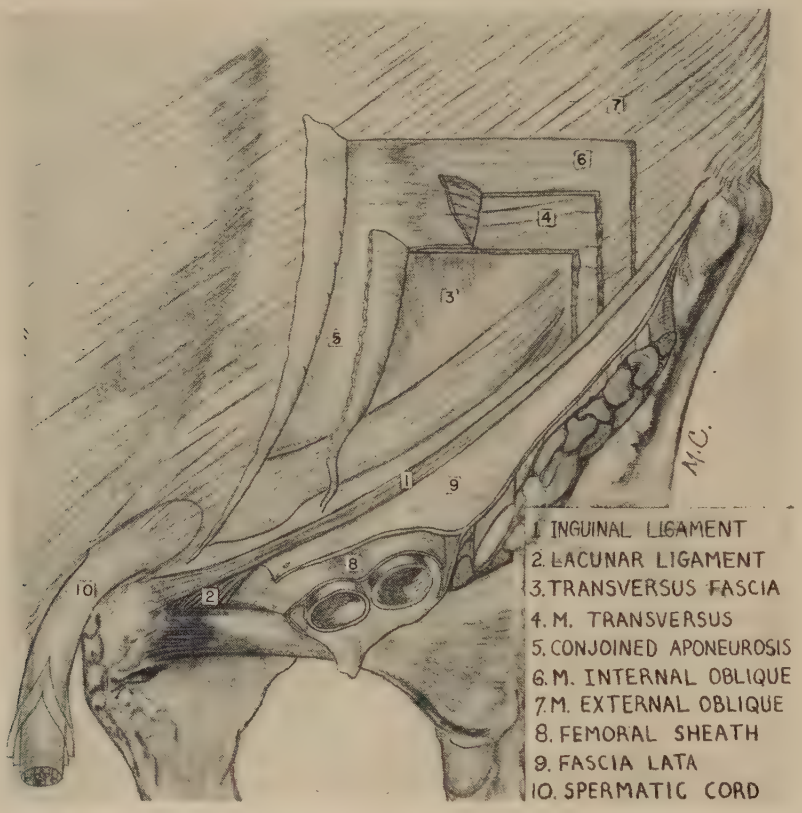

Fig. 2 The inguino-hypogastric region in the adult. The origin of the three fascial layers is shown. The proximal portion of the spermatic cord has been deflected upward to show the fibers of the lacunar ligament which run parallel with remainder of the inguinal ligament.

DISCUSSION

Hernias of various sizes have been encountered in this small series. The presence of even a dimpling of the peritoneum is usually considered as a necessary precursor of indirect

inguinal hernia. It has been estimated (Fitch, '31) from meager autopsy and operating room reports that nearly 10% of the population has at least a small amount of the original peritoneal protrusion into the inguinal canal.

The question is often asked, when does a protrusion become a hernia? Probably when it enlarges sufficiently to produce pain or some other indication of its presence. The presence of a viscus does not seem to be necessary. In the smallest protrusion in this series the sac had entered the internal ring but when observed did not contain a viscus. The largest hernial sac contained much of the jejunum, ileum and transverse colon. Hernias of this size distort the deep inferior epigastric vessels and render the inguinal canal a straight passageway.

Although the number of fetuses studied is not large enough to justify a statistical study of the rate of growth of the processus vaginalis it certainly does not bear out Frankl's ('00) statement that the processus is 1 mm in length at the 5th lunar month or that of Wells ('43) who says no evagination was observed until the 6th lunar month.

Histologically the gubernaculum from 10 fetuses (16 to 28 cm crown-rump length) is or closely resembles mucoid connective tissue. Forssner ('28) regards it as a structure which enlarges the inguinal canal so that the testis can pass down to the scrotum. As soon as this enlargement has taken place the gubernaculum becomes soft and loses all sustaining power. The testes then sink down through the gubernaculum and inguinal canal into the scrotum. In all instances of this series the gubernaculum was less than one-third the size of the testis. Furthermore, the testes were never located in the distal end of the processus vaginalis until they reached the bottom of the scrotum.

Wells regards the gubernaculum as important in that it contributes to the development and expansion of the processus. This study does not include material at the time of the formation of the processus but since the diameter is only approximately one-third that of the testis, it is hard to see

how it could aid in the expansion of the processus. Wells ('44) also refers to the "steering" influence of the gubernaculum on the testis. Since it seems to be composed of mucoid connective tissue it might not exert traction but still act as a guide. It is hard to see how the gubernaculum could exert traction, since it is contained within the processus vaginalis and does not become anchored until it reaches the scrotum. Furthermore, since the gubernaculum is attached to the entire extent of the processus it does not seem reasonable that it could exert traction in any direction.

The rate of testicular descent as indicated by the data given above is greater than that reported by Wells. This discrepancy may be partly accounted for by the fact that Wells was using some fresh material. In fixed tissue, different degrees of shrinkage may be encountered.

CONCLUSIONS

These observations on the inguinal canal in man are based on dissections of 290 body halves and 36 fetuses. The gubernacula in 10 fetuses were studied histologically.

The inguinal canal is the result of the evagination of the peritoneum and fascial protrusions of the transverse fascia, conjoined aponeurosis and aponeurosis of the external oblique. In the male the testes pass through this evagination to the scrotum and thus the spermatic cord becomes surrounded by fascial layers derived from the abdominal wall. All of the peritoneal protrusion normally disappears except the distal portion which is the tunica vaginalis (spermatic sac) of the testis. If a foveate depression remains in the original protrusion at the internal ring, it constitutes a predisposing factor to indirect inguinal hernia. It seems likely that in the complete absence of a dimpling of the peritoneum in the lateral inguinal fossa, indirect inguinal hernias do not occur.

The growth of the ventral abdominal wall renders the inguinal canal oblique. If a remnant of the peritoneal protrusion persists in the internal ring, changes in intra-abdom-

inal pressure may cause it to enlarge. Any increase in diameter of the internal ring reduces by a corresponding amount the obliquity of the canal. When the two rings are superimposed upon each other the inguinal triangle is obliterated and the inguinal canal converted to an open passageway. In this condition the hernial sac rests upon the deep inferior epigastric vessels.

Definite sharp margins are produced in the transversus fascia and the aponeurosis of the external oblique which are known as "rings." The size of the rings are often considered predisposing factors to hernia. This might be true of the internal ring but even here the necessary predisposing factor seems to be a persistent foveate depression rather than the size of the ring. The processus was near 1 mm in length in fetuses about 4 months old and about 2 cm in those near 7 lunar months.

The gubernaculum is or closely simulates mucoid connective tissue. It is approximately one-third the size of the testis so that it probably does not expand the diameter of the processus vaginalis or exert traction on the testis. In no instance could any portion be found extending outside the processus vaginalis toward or into the scrotum.

When first formed the inguinal canal is short and straight. The growth of the ventral abdominal wall brings the internal ring laterally and thus gives obliquity to the canal. Any enlargement of a persistent foveate depression in the inguinal canal both shortens and straightens the canal.

In this series 20 indirect inguinal hernias were encountered. The smallest one did not contain a viscus at all but the largest one contained a large part of both the small and the large intestine.

SUMMARY

1. The inguinal canal results from the formation of the processus vaginalis in fetal life.

2. In the adult the canal is oblique because growth of the body wall shifts the internal ring laterally.

3. An indirect inguinal hernia is the enlargement of a protrusion of the peritoneum into the internal abdominal ring.

4. A hernial protrusion may not contain anything. It may be large enough to hold a large part of the small and large bowel.

5. The gubernaculum is apparently mucoid connective tissue. It probably does not extend the processus vaginalis or exert traction on the testis.

ACKNOWLEDGMENTS

I am indebted to Miss Helen Hess and to Miss Martha Coyner who made the illustrations. I am grateful to the Anatomy Departments at Pittsburgh, Northwestern, Chicago and Columbia, who made it possible for me to study cadavers at the medical schools. Doctor George W. Corner generously gave me permission to dissect the inguinal region in 33 fetuses.

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BOOK REVIEW

THE MAMMALIAN ADRENAL GLAND. By Geoffrey H. Bourne. 1949. vii + 239 pages and 15 plates, $6.00. The Clarendon Press, Oxford.

No worker in the endocrine field with special interest in the adrenal should be without this book. It furnishes excellent basic material on the gross structure, size, and microscopic anatomy of the adrenal in many species of mammals and in addition includes a lucid discussion of the adrenal throughout the vertebrates.

In an historical summary written in a readable running style the author does not hesitate to make side excursions into recent developments while elaborating upon a particular theme.

The adrenals in the vertebrates first appear as rudiments in the Cyclostomes, the cortical precursor being separate from the chromaffine tissue. In higher vertebrates the gland becomes more complex until it reaches the form found in mammals. A classification of mammals lists those species which have been studied and gives the author and the date of the publication concerned with that species. Over half the original descriptions in this book have been furnished for the first time by the author.

Three chapters are devoted to descriptions of adrenal structure in the Monotremata, the Metatheria, and the Eutheria. The relationship between placentation and the organization of the adrenal is developed in this discussion. Egg-laying mammals do not have the highly organized form. The final chapter is an excellent general summary of our knowledge of mammalian adrenals in which anatomical features are correlated with function. An extensive bibliography, an index, and 15 half-tone plates complete the volume.

An interesting final conclusion is that the remarkable constancy of the mammalian adrenal leads the author to believe that "an adrenal gland in which the medulla is in the main completely separated from the cortex and which is surrounded completely or for three-quarters of its circumference by the latter is as diagnostic of the Mammalia as hair and nails."

Raymund L. Zwemer